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(54) Title: A DETERGENT COMPOSITION COMPRISING TWO CELLULASE COMPONENTS

(57) Abstract

Detergent compositions comprising 1) a first cellulase component having retaining-type activity, preferably having a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1} and being capable of particulate soil removal, and 2) a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain, preferably having a catalytic activity on Red Avicel 7.5 per 1 mg of cellulase protein higher than 10^{-4} IU and being capable of colour clarification, wherein at least one of the cellulase components is a single (recombinant) component, are useful for cleaning and colour clarification of cellulose-containing fabrics.

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A detergent composition comprising two cellulase components.

The present invention relates to a detergent composition comprising cellulases which is capable of providing improved particulate soil removal as well as colour clarification when used for washing cellulose containing fabrics.

BACKGROUND OF THE INVENTION

Repeated washing of fabrics, especially cellulose containing fabrics, generally causes a harshness in the fabric used. The use of cellulolytic enzymes for harshness reduction of cellulose containing fabrics, e.g. cotton, was suggested and demonstrated a long time ago. However, the mechanism of this reduction has not yet been elucidated in detail.

The need for detergent compositions which exhibit not only good cleaning properties, but also good fabric-softening performance, and other fabric care benefits, is now well-established in the art.

In the patent application WO 89/09259 (Novo Industri A/S) a cellulase preparation to be used for reducing the harshness of cotton-containing fabrics has been described. In this patent application, WO 89/09259, is disclosed a cellulase fraction enriched in endoglucanase activity.

The efficiency of cellulolytic enzymes, i.e. cellulases, in terms of textile cleaning and harshness-reducing agent for fabrics has been recognized for some time; GB-A-2,075,028, GB-A-2,095,275 and GB-A-2,094,826, disclose detergent compositions with cellulase for improved cleaning performance; GB-A-1,368,599 discloses the use of cellulase for reducing the harshness of cotton-containing fabrics; U.S. 4,435,307 teaches the use of a cellulolytic enzyme derived from Humicola insolens as well as a fraction thereof, designated ACXI, as a harshness-reducing detergent additive.

EP-A-0 269 168 discloses optimized detergent compositions containing cellulase, which are formulated at a mild alkaline pH range and provide combined fabric cleaning, fabric softening, and fabric care performance.

The practical exploitation of cellulases has been set back by the fact that the cellulase preparations known in the art are complex mixtures of which only a certain fraction is effective in the fabric-care context; ; it was thus difficult to implement cost effective industrial production of cellulase for the detergent industry; and large quantities of such cellulase preparations would need to be applied, in order to obtain the desired effect on fabrics.

Improvements in cellulase production also often have not proven to be sufficiently identifiable in terms of applicability in detergents.

Until present it has been a problem to relate the beneficial action of certain enzyme preparations such as cellulases for laundry wash unambiguously to the internationally accepted enzyme classification. For example, many enzymes described as cellulases which, according to all the hitherto known criteria, would be expected to exhibit good washing performance are not active in respect of colour clarification on cellulose containing fabrics under washing conditions.

At present, the only guideline available for the selection of appropriate enzymes capable of performing good washing performance and colour clarification is full scale washing trials which make heavy demands on time and resources. All the known assays for evaluating cellulase activity such as using CMC, filter paper, amorphous and crystalline cellulose are not able to distinguish valuable enzymes from inactive enzymes and do not provide any suggestions regarding the expected performance when used for washing cellulose containing fabrics.

Thus, it is a problem to develop more efficient cellulase-containing detergent compositions which satisfy the customer needs, since it is not known in the state of the art which kind of cellulase enzymes is actually functioning for this purpose.

Also, it is desirable to provide novel enzymatic detergent compositions capable of providing both sufficient colour clarification and particulate soil removal which, after a limited number of washing cycles, neither damage nor partly degrade the cellulose-containing fabric, e.g. the cotton.

SUMMARY OF THE INVENTION

The present invention relates to detergent compositions comprising a first cellulase component having retaining-type activity and being capable of particulate soil removal and a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and being capable of colour clarification wherein at least one of the cellulase components is a single (recombinant) component.

Such compositions are particularly useful as laundry detergents, both granular as well as liquid detergents.

Surprisingly, it has been found that the cellulase component which is active in respect of colour clarification when used for washing cellulose-containing fabrics, preferably has multiple domains, i.e. one or more catalytic domains attached to one or more non-catalytic domains, e.g. cellulose binding domains, and that the component may have retaining-type activity or inverting-type activity; and that the cellulase component which is active in respect of particulate soil removal when used for washing cellulose-containing fabrics, has retaining-type activity.

It is believed that the retaining-type activity of the first cellulase component may be demonstrated by the capability of the component to exhibit catalytic activity on low molecular weight carbohydrate substrates; and that the multiple domain architecture of the second cellulase component capable of colour clarification may be demonstrated by the capability of the component to exhibit high catalytic activity on cellodextrins, especially cellodextrins having 6 glucose units (DP6), e.g. dyed microcrystalline cellulose, and essentially no catalytic activity on low molecular weight carbohydrate substrates.

It has also been found that a cellulase composition consisting of at least two cellulolytic components, the first component exhibiting a low degree of activity towards dyed microcrystalline cellulose and a high degree of activity towards short cellooligosaccharides and the second component exhibiting a high degree of activity towards dyed microcrystalline cellulose, may be used complementary in detergent compositions for the improvement of the performance of detergents used for washing cellulose-containing fabrics, e.g. cotton, in particular for achieving particulate soil removal (first component) and better colour clarification (second component) without inducing fabric damage.

The invention further relates to detergent compositions having said first and second cellulase components with above-mentioned benefits together with improved stability in heavy duty liquids in the presence of proteases. It has previously been observed that cellulases are sensitive to the action of proteases, i.e. that in the presence of proteases commonly employed in detergents, cellulases are degraded to lower molecular weight polypeptides resulting in inactivation of the cellulase enzymes in question.

The first cellulase component of the composition according to the invention exhibits surprisingly and totally unexpected a high stability of the performance activity in a

neutral pH of heavy duty liquid detergent compositions with high level of detergent protease. The performance stability of this cellulase component has been found to be less susceptible to degradation by protease in a heavy duty liquid composition with conventional boric acid based reversible protease inhibitors.

The composition of the neutral pH heavy duty liquid can be widely varied in terms of surfactant composition, levels of the protease and protease reversible inhibitors without losing the primary advantage of the invention. Typical examples of detergent compositions according to the invention which comprise the mentioned first and second cellulase components are described in the Example Section of this Application.

Another object of the present invention is to provide a detergent additive comprising a first cellulase component capable of particulate soil removal and a second cellulase component capable of colour clarification wherein at least one of the cellulase components is a single component.

It is yet another object of the present invention to provide a method for treating fabrics in a washing machine comprising the utilization of the present detergent composition.

THE DRAWINGS

The present invention is further illustrated by the drawings in which

Figure 1 shows the mechanism of a retaining glycosidase; and

Figure 2 shows the mechanism of an inverting glycosidase.

DETAILED DESCRIPTION OF THE INVENTION

In the present specification and claims, the term "cellulase component" denotes an enzyme that hydrolyses cellulose. The cellulase component may be a component occurring in a cellulase system produced by a given microorganism, such a cellulase system mostly comprising several different cellulase enzyme components including those usually identified as e.g. cellobiohydrolases, exo-cellobiohydrolases, endoglucanases, β-glucosidases.

Alternatively, the cellulase component may be a single component, i.e. a component essentially free of other cellulase components usually occurring in a cellulase system produced by a given microorganism, the single component being a recombinant component, i.e. produced by cloning of a DNA sequence encoding the single component and subsequent cell transformed with the DNA sequence and expressed in a host, cf. e.g. International Patent Applications WO 91/17243 and WO 91/17244 which are hereby incorporated by reference. The host is preferably a heterologous host, but the host may under certain conditions also be the homologous host.

As used herein, the term "weight of cellulase protein" denotes the weight of the protein constituting a cellulase component.

The term "colour clarification", as used herein, refers to preservation of the initial colours throughout multiple washing cycles by removing fuzz and pills from the surface of garment and/or fabric.

The term "particulate soil removal", as used herein, refers to enhanced cleaning of cellulose-containing fabrics or garment, e.g. cotton, contaminated by particles of soil or of other insoluble matter entrapped by microfibrills spreading out on the fibre surface.

The term "retaining-type activity", as used herein, is intended to mean the stereochemical course of hydrolysis catalysed by a (first) cellulase component wherein the mechanism (of a retaining glycosidase) is as shown in Figure 1, cf. *Chem. Rev., 90, p. 1171-1202 (1990) (Sinott, M.L.:* Catalytic mechanism of enzymatic glycosyl transfer). Both the cleavage product leaving the active site of the cellulase having retaining-type activity as well as the substrate is in β-configuration, cf. *Eur. J. Biochem, 217, p. 947-953 (1993).*

The term "inverting-type activity", as used herein, is intended to mean the stereochemical course of hydrolysis catalysed by a cellulase component wherein the mechanism (of an inverting glycosidase) is as shown in Figure 2, cf. *Chem. Rev., 90, p. 1171-1202 (1990) (Sinott, M.L.:* Catalytic mechanism of enzymatic glycosyl transfer) and *Eur. J. Biochem, 217, p. 947-953 (1993).*

The stereochemistry of hydrolysis of the glycosidic bond is firmly dictated by the structure and topology of the enzyme active site and is usually interpreted as the result of a single-displacement or double displacement catalytic mechanism. It is believed that all enzymes in a given cellulase family, cf. *Gene (Amst.), 81, p. 83-95 (1989)* and *Biochem. J., 293, p. 781-788 (1993)*, have a similar fold even when their amino acid conservation is extremely low, and it is furthermore shown that members of a given cellulase family all have the same general fold and topology (*J. Biochem, 217, p. 947-953 (1993)*).

Furthermore, it is contemplated that the first cellulase component may have an exo-mode of action, the term "exo-mode of action" being intended to mean initiating degradation of cellulose from the non-reducing chain ends by removing cellobiose units.

Also, it is contemplated that the second cellulase component may have an endo-mode of action, the "endo-mode of action" being intended to mean hydrolysing amorphous regions of low crystallinity in cellulose fibres.

The term "domain", as used herein, is intended to indicate an amino acid sequence capable of effecting a specific task. For example is the term "carbohydrate binding domain" or "cellulose binding domain" ("CBD") intended to indicate an amino acid sequence capable of effecting binding of the enzyme to a carbohydrate substrate, in particular cellulose, and the term "catalytic active domain" ("CAD") is intended to indicate an amino sequence capable of effecting catalytic cleavage and having one or more active sites. A CBD is an example of a non-catalytic domain. CAD's and CBD's may be linked or attached by linking regions. Cf. *Trends Biotechnol., 5, p. 255-261 (1987)* and *Microbiol. Rev., 55, p. 303-315 (1991)*.

The term "core enzyme", as used herein, is intended to indicate an enzyme consisting essentially of a single domain, i.e. a catalytic active domain, the core enzyme having no "tail".

The term "activity towards dyed microcrystalline cellulose" as used herein refers to a hydrolytic activity towards microcrystalline cellulose covalently labelled with a light absorbing/fluorogenic compound, e.g. a reactive dye, determined spectroscopically by measuring the liberation of labelled products resulting from hydrolysis under conditions simulating washing conditions with respect to alkaline pH, temperature, duration, agitation and detergent concentrations. The assay is described below under "Methods".

Accordingly, a cellulase component exhibiting catalytic activity towards dyed microcrystalline cellulose must be active in releasing labelled soluble products from modified microcrystalline cellulose under simulated washing conditions.

The term "activity towards short cellooligosaccharides" as used herein, refers to an activity towards cellooligosaccharides containing two glucose units and an additional leaving group, such as e.g. a glucose unit, or a modified glucose unit, or a chromogenic/fluorogenic group, or other groups, resulting in splitting the glycosidic bond and measured as reducing end recovery or chromogenic or fluorogenic label compound liberation under hydrolysis under conditions simulating washing conditions with respect to alkaline pH, temperature, duration, agitation and detergent concentrations. The assay is described below under "Methods".

Accordingly, a cellulase component exhibiting a catalytic activity towards short cellooligosaccharides must be active in hydrolysis of short cellooligosaccharides under washing conditions, the cellooligosaccharides containing two glucose units and an additional leaving group, such as e.g. a glucose unit, or a modified glucose unit, or a chromogenic/fluorogenic group, or other groups.

In the present context, the term "immunoreactive" is intended to indicate that the produced protein is reactive with an antibody raised against a native cellulose- or hemicellulose-degrading enzyme.

In the present context, the term "homologue" is intended to indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for the cellulase component with the amino acid sequence in question under certain specified conditions (such as presoaking in 5xSSC and prehybridising for 1 h at ~40°C in a solution of 20% formamide, 5xDenhard´t's solution, 50 mM sodium phosphate, pH 6.8, and 50 µg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 µM ATP for 18 h at ~40°C). The term is intended to include derivatives of the sequence in question obtained by addition of one or more amino acid residues to either or both the C- and N-terminal of the native sequence substitution of one or

mor amino acid residues at one or more sites in the native sequence, deletion of one or more amino acid residues at either or both ends of the native amino acid sequence or at one or more sites within the native sequence, or insertion of one or more amino acid residues at one or more sites in the native sequence. It is to be understood that any derivative also hybridizes to the same probe as mentioned above which indicates that the cellulase enzyme derivatives within the scope of the present invention all have the same advantageous activity and effect as the cellulase component having the amino acid sequence in question. Also, any additions or substitutions or deletions or insertions may preferably relate to a relatively limited number of amino acids of the sequence in question, i.e. minor additions, substitutions, deletions or insertions, since it is to be expected that major additions, substitutions, deletions or insertions may result in cellulase components (polypeptides) which do not fulfil the above-mentioned hybridizing requirement.

The present invention relates to a detergent composition comprising a first cellulase component having retaining-type activity and being capable of particulate soil removal and a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and being capable of colour clarification wherein at least one of the cellulase components is a single (recombinant) component.

The cellulase components may be obtained from the microorganism in question by use of any suitable technique. For instance, a cellulase preparation may be obtained by fermentation of a microorganism and subsequent isolation of a cellulase containing preparation from the fermented broth or microorganism by methods known in the art, but more preferably by use of recombinant DNA techniques as known in the art. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the cel-

lulase component in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture.

CLONING A DNA SEQUENCE ENCODING A CELLULASE

The DNA sequence encoding a parent cellulase may be isolated from any cell or microorganism producing the cellulase in question by various methods, well known in the art. First a genomic DNA and/or cDNA library should be constructed using chromosomal DNA or messenger RNA from the organism that produces the cellulase to be studied. Then, if the amino acid sequence of the cellulase is known, homologous, labelled oligonucleotide probes may be synthesized and used to identify cellulase-encoding clones from a genomic library of bacterial DNA, or from a fungal cDNA library. Alternatively, a labelled oligonucleotide probe containing sequences homologous to cellulase from another strain of bacteria or fungus could be used as a probe to identify cellulase-encoding clones, using hybridization and washing conditions of lower stringency.

Yet another method for identifying cellulase-producing clones would involve inserting fragments of genomic DNA into an expression vector, such as a plasmid, transforming cellulase-negative bacteria with the resulting genomic DNA library, and then plating the transformed bacteria onto agar containing a substrate for cellulase. Those bacteria containing cellulase-bearing plasmid will produce colonies surrounded by a halo of clear agar, due to digestion of the substrate by secreted cellulase.

Alternatively, the DNA sequence encoding the enzyme may be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by S.L. Beaucage and M.H. Caruthers, Tetrahedron Letters 22, 1981, pp. 1859-1869, or the method described by Matthes et al., The EMBO J. 3, 1984, pp. 801-805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

Finally, the DNA sequence may be of mixed genomic and synthetic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA sequence, in accordance with standard techniques. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., Science 239, 1988, pp. 487-491.

EXPRESSION OF CELLULASE VARIANTS

According to the invention, a mutated cellulase-coding sequence produced by methods described above, or any alternative methods known in the art, can be expressed, in enzyme form, using an expression vector which typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal, and, optionally, a repressor gene or various activator genes. To permit the secretion of the expressed protein, nucleotides encoding a "signal sequence" may be inserted prior to the cellulase-coding sequence. For expression under the direction of control sequences, a target gene to be treated according to the invention is operably linked to the control sequences in the proper reading frame. Promoter sequences that can be incorporated into plasmid vectors, and which can support the transcription of the mutant cellulase gene, include but are

not limited to the prokaryotic ß-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731) and the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25). Further references can also be found in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94.

According to one embodiment B. subtilis is transformed by an expression vector carrying the mutated DNA. If expression is to take place in a secreting microorganism such as B. subtilis a signal sequence may follow the translation initiation signal and precede the DNA sequence of interest. The signal sequence acts to transport the expression product to the cell wall where it is cleaved from the product upon secretion. The term "control sequences" as defined above is intended to include a signal sequence, when is present.

In a currently preferred method of producing cellulase variants of the invention, a filamentous fungus is used as the host organism. The filamentous fungus host organism may conveniently be one which has previously been used as a host for producing recombinant proteins, e.g. a strain of Aspergillus sp., such as A. niger, A. nidulans or A. oryzae. The use of A. oryzae in the production of recombinant proteins is extensively described in, e.g. EP 238 023.

For expression of cellulase variants in Aspergillus, the DNA sequence coding for the cellulase variant is preceded by a promoter. The promoter may be any DNA sequence exhibiting a strong transcriptional activity in Aspergillus and may be derived from a gene encoding an extracellular or intracellular protein such as an amylase, a glucoamylase, a protease, a lipase, a cellulase or a glycolytic enzyme.

Examples of suitable promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral α-amylase, A. niger acid stable α-amylase, A. niger glucoamylase, Rhizomucor

miehei lipase, A. oryzae alkaline protease or A. oryzae triose phosphate isomerase.

In particular when the host organism is A. oryzae, a preferred promoter for use in the process of the present invention is the A. oryzae TAKA amylase promoter as it exhibits a strong transcriptional activity in A. oryzae. The sequence of the TAKA amylase promoter appears from EP 238 023.

Termination and polyadenylation sequences may suitably be derived from the same sources as the promoter.

The techniques used to transform a fungal host cell may suitably be as described in EP 238 023.

To ensure secretion of the cellulase variant from the host cell, the DNA sequence encoding the cellulase variant may be preceded by a signal sequence which may be a naturally occurring signal sequence or a functional part thereof or a synthetic sequence providing secretion of the protein from the cell. In particular, the signal sequence may be derived from a gene encoding an Aspergillus sp. amylase or glucoamylase, a gene encoding a Rhizomucor miehei lipase or protease, or a gene encoding a Humicola cellulase, xylanase or lipase. The signal sequence is preferably derived from the gene encoding A. oryzae TAKA amylase, A. niger neutral α-amylase, A. niger acid-stable α-amylase or A. niger glucoamylase.

The medium used to culture the transformed host cells may be any conventional medium suitable for growing Aspergillus cells. The transformants are usually stable and may be cultured in the absence of selection pressure. However, if the transformants are found to be unstable, a selection marker introduced into the cells may be used for selection.

The mature cellulase protein secreted from the host cells may conveniently be recovered from the culture medium by

well-known procedures including separating the cells from the medium by centrifugation or filtration, and precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

The component comprised by the detergent composition of the invention which is not a single recombinant component may be a component produced by conventional techniques such as produced by a given microorganism as a part of a cellulase system.

In a preferred embodiment of the invention, the single component produced by cloning and expression in a heterologous host is present in the detergent composition in an amount of at least 5%, preferably at least 10%, especially at least 20%, based on the total weight of cellulase protein in the composition.

Both the first and the second component may be recombinant (single) components, respectively, i.e. produced by cloning of the DNA sequence encoding the single component and cell transformation with the DNA sequence and expression in a host which may be heterologous or homologous. However, the first and second component may also be cloned and expressed in the same heterologous or homologous host.

In a preferred embodiment of the invention, the first and the second cellulase component are present in the detergent composition in a weight ratio of cellulase protein preferably in the range from about 30:1 to about 1:30, more preferably in the range from about 10:1 to about 1:10, especially in the range from about 2:1 to 1:2.

Accordingly, the detergent composition claimed in the present invention should preferably comprise the first and the second cellulase component, respectively, in a concentration

corresponding to a concentration in the resulting washing liquor of 0.001 - 100 mg of cellulase protein per litre of washing liquor.

Preferably, the first and the second cellulase component, respectively, is a fungal or bacterial cellulase component, i.e. of fungal or bacterial origin.

It is contemplated that first and second cellulase components, respectively, may be derived or isolated and purified from microorganisms which are known to be capable of producing cellulolytic enzymes, e.g. species of Humicola, Bacillus, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Schizophyllum, Penicillium, Aspergillus, and Geotricum. The derived components may be either homologous or heterologous components. Preferably, the components are homologous. However, a heterologous component which is immunoreactive with an antibody raised against a highly purified cellulase component possessing the desired property or properties and which heterologous component is derived from a specific microorganism is also preferred.

Preferably, the first cellulase exhibits catalytic activity on low molecular weight carbohydrate substrates, especially a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1} .

The first cellulase component may be inadequate or unable of providing colour clarification, thus exhibiting low catalytic activity on dyed microcrystalline cellulose.

In a preferred embodiment of the invention, the first cellulase component is a core enzyme, i.e. a cellulase having no "tail" or being a single domain protein.

A convenient first cellulase component useful in the detergent composition of the present invention may be a cellobiohydrolase component which is immunoreactive with an antibody

raised against a highly purified ~70kD cellobiohydrolase (EC 3.2.1.91) derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the ~70kD cellobiohydrolase exhibiting cellulase activity. A preferred cellobiohydrolase component has the amino acid sequence disclosed in *Nucleic Acid Research, vol. 18 (1990), page 668 (De Oliviera, Alzevedo, M. and Radford, A.)* which is shown in the appended SEQ ID NO:1 or a variant of said cellobiohydrolase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the cellobiohydrolase component is referred to as CBH I.

Another preferred cellobiohydrolase component is a core enzyme ("core CBH I") having an amino acid sequence consisting of 449 amino acids corresponding to the (partial) amino acid sequence numbered 1-449 of the appended SEQ ID NO:1. The core CBH I has an apparant molecular weight of ~48 kD.

Alternatively, the first cellulase component may be an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~50kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the ~50kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO91/17244, Fig. 14A-E, which is shown in the appended SEQ ID NO:2, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the endoglucanase component is referred to as EG I.

Alternatively, the first cellulase component may be an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~50kD (apparant molecular

weight, the amino acid composition corresponds to 45kD with 2n glycosylation sites) endoglucanase derived from *Fusarium oxysporum*, DSM 2672, or which is a homologue or derivative of the ~50kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO91/17244, Fig. 13, which is shown in the appended SEQ ID NO:3, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the endoglucanase component is referred to as EG I-F.

The EG I-F cellulase component is producible by *Aspergillus oryzae* after transformation with a plasmid containing the DNA sequence corresponding to the amino acid sequence of the appended SEQ ID NO:3 and using the conventional Taka promotor and AMG terminator. The EG I-F may be purified to homogeneity using cationic chromatography and has a pI >9. The calculated pI is 9 based on the amino acid composition using the PHKa values from *Adv. Protein Chem. 17, p. 69-165 (1962)(C. Tanford)*. The molar exctinction coefficient is calculated to be 58180.

Yet another preferred first cellulase component may be any of the cellulases disclosed in the published European Patent Application No. EP-A2-271 004, the cellulase having a non-degrading index (NDI) of not less than 500 and being an alkalophilic cellulase having an optimum pH not less than 7 or whose relative activity at a pH of not less than 8 is 50% or over of the activity under optimum conditions when carboxy methyl cellulose (CMC) is used as a substrate; the cellulase preferably being selected from the group consisting of alkaline cellulase K (produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase K-534 (produced by Bacillus sp. KSM-534, FERM BP 1508); alkaline cellulase K-539 (produced by Bacillus sp. KSM-539, FERM BP 1509); alka-

line cellulase K-577 (produced by Bacillus sp. KSM-577, FERM BP 1510); alkaline cellulase K-521 (produced by Bacillus sp. KSM-521, FERM BP 1507); alkaline cellulase K-580 (produced by Bacillus sp. KSM-580, FERM BP 1511); alkaline cellulase K-588 (produced by Bacillus sp. KSM-588, FERM BP 1513); alkaline cellulase K-597 (produced by Bacillus sp. KSM-597, FERM BP 1514); alkaline cellulase K-522 (produced by Bacillus sp. KSM-522, FERM BP 1512); CMCase I, CMCase II (both produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase E-II and alkaline cellulase E-III (both produced by Bacillus sp. KSM-522, FERM BP 1512).

Preferably, the second cellulase component being capable of colour clarification has multiple domains, i.e. one or more catalytic domains attached to one or more non-catalytic domains, e.g. cellulose binding domains, since the activity in respect of colour clarification is enhanced by the presence of e.g. a cellulose binding domain.

The second cellulase component may have retaining-type activity or inverting-type activity.

Preferably, the second cellulase component exhibits high catalytic activity on cellodextrin(s), more preferably on relatively long-chained cellodextrin(s), especially on reduced longer-chained cellodextrin(s).

In a preferred embodiment of the invention, the second cellulase component exhibits high catalytic activity on dyed microcrystalline cellulose, especially a catalytic activity on Red Avicel per 1 mg of cellulase protein higher than 10^{-4} IU, see below under "Methods" for the definitions of 1 IU of enzyme activity.

(Second) Cellulase components useful as colour clarifying components in the detergent composition of the present invention usually exhibits essentially no catalytic activity on low molecular weight carbohydrate substrates. Preferably,

the second cellulase component has a catalytic activity on low molecular weight carbohydrate substrates, especially on cellotriose, at pH 8.5 corresponding to k_{cat} of below 0.01 s^{-1}; more preferably the second cellulase component exhibits essentially no catalytic activity on cellotriose, i.e. the component is not capable of hydrolysing cellotriose but capable of hydrolysing higher oligomers of β-1,4-glucose units.

The catalytic activity on Red Avicel may be measured as described below under "Methods".

Although the main purpose of the presence of the second cellulase component in the detergent composition of the invention is the colour clarifying capability of the component, the second component may often also be capable of particulate soil removal.

A convenient second cellulase component useful in the detergent composition of the present invention may be an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~43kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the ~43kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO 91/17243, SEQ ID#2, which is shown in the appended SEQ ID NO:4, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the endoglucanase component is referred to as EG V.

Another preferred endoglucanase component comprises an amino acid sequence encoded by the partial DNA sequence disclosed in PCT Patent Application No. WO93/11249; SEQ ID#11, which is shown in the appended SEQ ID NO:5, or a variant of said

endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the endoglucanase component is referred to as EG VI.

Yet another preferred endoglucanase component comprises an amino acid sequence encoded by the partial DNA sequence disclosed in PCT Patent Application No. WO 93/11249, SEQ ID#9, which is hereby incorporated by reference. In example 1 below, the endoglucanase component is referred to as EG II.

Yet another preferred endoglucanase component comprises an amino acid sequence encoded by the partial DNA sequence disclosed in PCT Patent Application No. WO93/11249, SEQ ID#7, which is hereby incorporated by reference. In example 1 below, the endoglucanase component is referred to as EG III.

Alternatively, the second cellulase component may be an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~60kD endoglucanase derived from *Bacillus lautus,* NCIMB 40250, or which is a homologue or derivative of the ~60kD endoglucanase exhibiting cellulase activity. A preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO 91/10732, SEQ ID#7, which is shown in the appended SEQ ID NO:6, or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous with said sequence. In example 1 below, the ~60kD endoglucanase component is referred to as EG C.

In a specific aspect, the invention provides a detergent additive. The enzymes may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a

separated additive or a combined additive, can be formulated e.g. as granulates, liquids, slurries, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, slurries, or protected enzymes.

Dust free granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally be coated by methods known in the art. The detergent enzymes may be mixed before or after granulation.

Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid, boric acid or a boric acid derivative, e.g. an aromatic borate ester, and the preparation may be formulated according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238 216.

DETERGENT COMPOSITIONS

The detergent composition of the invention may be formulated in any convenient form, e.g. as a powder or liquid. Detergent compositions of the invention may contain other detergent ingredients known in the art as e.g. builders, bleaching agents, bleach activators, anti soil redeposition agents, perfumes, etc. as shown in the examples.

Additionally detergent compositions comprise surfactants which may be of the anionic, non-ionic,amphoteric, cationic or zwitterionic type as well as mixtures of these types.

A typical listing of these surfactants is given in US Patent 3,664,961 issued to Norris on May 23, 1972.

Mixtures of anionic surfactants are particularly suitable herein, such as mixtures of sulphonate and sulphate surfactants in a weight ratio of from 5:1 to 1:2, preferably from 3:1 to 2:3, more preferably from 3:1 to 1:1. Preferred sulphonates include alkyl benzene sulphonates having from 9 to 15, especially 11 to 13 carbon atoms in the alkyl radical, and alpha-sulphonated methyl fatty acid esters in which the fatty acid is derived from a C_{12}-C_{18} fatty source preferably from a C_{16}-C_{18} fatty source. In each instance the cation is an alkali metal, preferably sodium. Preferred sulphate surfactants are alkyl sulphates having from 12 to 18 carbon atoms in the alkyl radical, optionally in admixture with ethoxy sulphates having from 10 to 20, preferably 10 to 16 carbon atoms in the alkyl radical and an average degree of ethoxylation of 1 to 6. Examples of preferred alkyl sulphates herein are tallow alkyl sulphate, coconut alkyl sulphate, and C_{14-15} alkyl sulphates. The cation in each instance is again an alkali metal cation, preferably sodium. Also preferred for use herein are mixtures of sulphates and/or ethoxysulphates.

One class of nonionic surfactants useful in the present invention are condensates of ethylene oxide with a hydrophobic moiety to provide a surfactant having an average hydrophilic-lipophilic balance (HLB) in the range from 8 to 17, preferably from 9.5 to 13.5, more preferably from 10 to 12.5. The hydrophobic (lipophilic) moiety may be aliphatic or aromatic in nature and the length of the polyoxyethylene group which is condensed with any particular hydrophobic group can be readily adjusted to yield a water-soluble compound having the desired degree of balance between hydrophilic and hydrophobic elements.

Especially preferred nonionic surfactants of this type are the C_9-C_{15} primary alcohol ethoxylates containing 3-8 moles of ethylene oxide per mole of alcohol, particularly the C_{14}-C_{15} primary alcohols containing 6-8 moles of ethylene oxide

per mole of alcohol and the C_{12}-C_{14} primary alcohols containing 3-5 moles of ethylene oxide per mole of alcohol.

Another class of nonionic surfactants comprises alkyl polyglucoside compounds of general formula

$$RO\ (C_nH_{2n}O)_tZ_x$$

wherein Z is a moiety derived from glucose; R is a saturated hydrophobic alkyl group that contains from 12 to 18 carbon atoms; t is from 0 to 10 and n is 2 or 3; x is from 1.3 to 4, the compounds including less than 10% unreacted fatty alcohol and less than 50% short chain alkyl polyglucosides. Compounds of this type and their use in detergent are disclosed in EP-B 0 070 077, 0 075 996 and 0 094 118.

Also suitable as nonionic surfactants are poly hydroxy fatty acid amide surfactants of the formula $R^2 - C(=O) - N(R^1) - Z$,

wherein R^1 is H, C_{1-4} hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R_2 is C_{5-31} hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R_1 is methyl, R_2 is a straight C_{11-15} alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

A further class of surfactants are the semi-polar surfactants such as amine oxides. Suitable amine oxides are selected from mono C_8-C_{20}, preferably C_{10}-C_{14} N-alkyl or alkenyl amine oxides and propylene-1,3-diamine dioxides wherein the remaining N positions are substituted by methyl, hydroxyethyl or hydroxypropyl groups.

Another class of surfactants are amphoteric surfactants, such as polyamine-based species.

Cationic surfactants can also be used in the detergent compositions herein and suitable quaternary ammonium surfactants are selected from mono C_8-C_{16}, preferably C_{10}-C_{14} N-alkyl or alkenyl ammonium surfactants wherein remaining N positions are substituted by methyl, hydroxyethyl or hydroxypropyl groups.

Mixtures of surfactant types are preferred, more especially anionic-nonionic and also anionic-nonionic-cationic mixtures. Particularly preferred mixtures are described in British Patent No. 2040987 and European Published Application No. 0 087 914. The detergent compositions can comprise from 1%-70% by weight of surfactant, but usually the surfactant is present in the compositions herein an amount of from 1% to 30%, more preferably from 10-25% by weight.

BUILDER

Builder materials will typically be present at from 5% to 80% of the detergent compositions herein. The compositions herein are free or substantially free of phosphate-containing builders (substantially free being herein defined to constitute less than 1% of the total detergent builder system), and the builder system herein consists of water-soluble builders, water-insoluble builders, or mixtures thereof.

Water insoluble builders can be an inorganic ion exchange material,commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated Zeolite A, X, B, MAP or HS.

Preferred aluminosilicate ion-exchange materials have the unit cell formula

$$M_z[(AlO_2)_z (SiO_2)_y] xH_2O$$

wherein M is a calcium-exchange cation, z and y are at least 6; the molar ratio of z to y is from 1.0 to 0.5 and x is at least 5, preferably from 7.5 to 276, more preferably from 10 to 264. The aluminosilicate materials are in hydrated form and are preferably crystalline containing from 10% to 28%, more preferably from 18% to 22% water.

The above aluminosilicate ion exchange materials are further characterized by a particle size diameter of from 0.1 to 10 micrometers, preferably from 0.2 to 4 micrometers. The term "particle size diameter" herein represents the average particle size diameter of a given ion exchange material as determined by conventional analytical techniques such as, for example, microscopic determination utilizing a scanning electron microscope. The aluminosilicate ion exchange materials are further characterized by their calcium ion exchange capacity, which is at least 200 mg equivalent of $CaCO_3$ water hardness/g of aluminosilicate, calculated on an anhydrous basis, and which generally is in the range of from 300 mg eq./g to 352 mg eq./g. The aluminosilicate ion exchange materials herein are still further characterized by their calcium ion exchange rate which is described in detail in GB-1,429,143.

Aluminosilicate ion exchange materials useful in the practice of this invention are commercially available and can be naturally occurring materials, but are preferably synthetically derived. A method for producing aluminosilicate ion exchange materials is discussed in US Patent No. 3,985,669. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designation Zeolite A, Zeolite B, Zeolite X, Zeolite MAP, Zeolite HS and mixtures thereof. In an especially preferred embodiment,

the crystalline aluminosilicate ion exchange material is Zeolite A and has the formula

$$Na_{12}[(AlO_2)_{12}\ (SiO_2)_{12}]\ xH_2O$$

wherein x is from 20 to 30, especially 27. Zeolite X of formula $Na_{86}[(AlO_2)_{86}(SiO_2)_{106}]-10.276H_2O$ is also suitable, as well as Zeolite HS of formula $Na_6[(AlO_2)_6(SiO_2)_6]$ 7.5 H_2O.

Another suitable water-insoluble, inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($Na_2Si_2O_5$). The high Ca^{++}/Mg^{++} binding capacity is mainly a cation exchange mechanism. In hot water, the material becomes more soluble.

The water-soluble builder can be a monomeric or oligomeric carboxylate chelating agent.

Suitable carboxylates containing one carboxy group include lactic acid, glycollic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycolic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenlegenschrift 2,446,686, and 2,446,687 and U.S. Patent No. 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829,

1,1,2,2-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates and 1,1,2,3-propane tetracarboxylates. Polycarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in U.S. Patent No. 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis,cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydrofuran - cis, cis, cis-tetracarboxylates, 2,5-tetrahydrofuran -cis - dicarboxylates, 2,2,5,5-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane -hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phtalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water-soluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for the purposes of the invention include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

OPTIONAL INGREDIENTS

The present compositions will typically include optional ingredients that normally form part of detergent compositions. Antiredeposition and soil suspension agents, optical brighteners, bleaches, bleach activators, suds suppressors, anticaking agents, dyes and pigments are examples of such optional ingredients and can be added in varying amounts as desired.

Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably from 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4^1-bis-(2-diethanolamino-4-

anilino -s- triazin-6-ylamino)stilbene-2:2^1 disulphonate, disodium 4, - 4^1-bis-(2-morpholino-4-anilino-s-triazin-6-ylaminostilbene-2:2^1 - disulphonate, disodium 4,4^1 - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2^1 - disulphonate, monosodium 4^1,4^{11} -bis-(2,4-dianilino-s-triazin-6 ylamino)stilbene-2-sulphonate, disodium 4,4^1 -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2^1 - disulphonate, disodium 4,4^1 -bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2^1 disulphonate, disodium 4,4^{1}bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2^{1}disulphonate sodium 2(stilbyl-4^{11}-(naphtho-1^1,2^1:4,5)-1,2,3 - triazole-2^{11}-sulphonate and disodium -4.4'-bis (2-sulfostyril)biphenyl.

Any particulate inorganic perhydrate bleach can be used, in an amount of from 3% to 40% by weight, more preferably from 8% to 25% by weight and most preferably from 12% to 20% by weight of the compositions. Preferred examples of such bleaches are sodium perborate monohydrate and tetrahydrate, percarbonate, and mixtures thereof.

Percarbonate particles for instance are dry-mixed with the other granular components of the detergent powder.

The compositions herein contain from 1 % to 40 %, preferably from 3 % to 30 % by weight, most preferably from 5 % to 25 % by weight of an alkali metal percarbonate bleach ; in the form of particles having a mean size from 250 to 900 micrometers, preferably 500 to 700 micrometers.

When the present compositions are laundry activities, the level of percarbonate is typically in the range of 20 % to 80 % by weight.

The alkali metal percarbonate bleach is usually in the form of the sodium salt. Sodium percarbonate is an addition compound having a formula corresponding to $2Na_2CO_3$ $3H_2O_2$. To enhance storage stability the percarbonate bleach can be

coated with a further mixed salt of an alkali metal sulphate and carbonate. Such coatings together with coating processes have previously been described in GB-1, 466, 799, granted to Interox on 9th March 1977. The weight ratio of the mixed salt coating material to percarbonate lies in the range from 1:2000 to 1:4, more preferably from 1:99 to 1:9, and most preferably from 1:49 to 1:19. Preferably, the mixed salt is of sodium sulphate and sodium carbonate which has the general formula $Na_2SO_4.n.Na_2CO_3$ wherein n is from 0.1 to 3, preferably n is from 0.3 to 1.0 and most preferably n is from 0.2 to 0.5.

Other suitable coating materials are sodium silicate, of SiO2:Na2O ratio from 1.6:1 to 2.8:1, and magnesium silicate.

Commercially available carbonate/sulphate coated percarbonate bleach may include a low level of a heavy metal sequestrant such as EDTA, 1-hydroxyethylidene 1,1-diphosphonic acid (HEDP) or an aminophosphonate, that is incorporated during the manufacturing process.
Preferred heavy metal sequestrants for incorporation as described herein above include the organic phosphonates and amino alkylene poly(alkylene phosphonates) such as the alkali metal ethane 1-hydroxy diphosphonates, the nitrilo trimethylene phosphonates, the ethylene diamine tetra methylene phosphonates and the diethylene triamine penta methylene phosphonates.

Especially when making a laundry detergent composition, the percarbonate-containing detergent powder preferably has a bulk density above 650 g/l.

Another preferred separately mixed ingredient is a peroxy carboxylic acid bleach percursor, commonly referred to as a bleach activator, which is preferably added in a prilled or agglomerated form. Examples of suitable compounds of this type are disclosed in British Patent Nos. 1586769 and 2143231 and a method for their formation into a prilled form

is described in European Published Patent Application No. 0 062 523. Preferred examples of such compounds are tetracetyl ethylene diamine and sodium 3, 5, 5 trimethyl hexanoyloxybenzene sulphonate.

Bleach activators are normally employed at levels of from 0.5% to 10% by weight, more frequently from 1% to 8% and preferably from 2% to 6% by weight of the composition.

Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can be generally represented by alkylated polysiloxane materials while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. These materials can be incorporated as particulates in which the suds suppressor is advantageously releasably incorporated in a water-soluble or water-dispersible, substantially non-surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

As mentioned above, useful silicone suds controlling agents can comprise a mixture of an alkylated siloxane, of the type referred to hereinbefore, and solid silica. Such mixtures are prepared by affixing the silicone to the surface of the solid silica. A preferred silicone suds controlling agent is represented by a hydrophobic silanated (most preferably trimethyl-silanated) silica having a particle size in the range from 10 millimicrons to 20 millimicrons and a specific surface area above 50 m^2/g intimately admixed with dimethyl silicone fluid having a molecular weight in the range from about 500 to about 200,000 at a weight ratio of silicone to silanated silica of from about 1:1 to about 1:2.

A preferred silicone suds controlling agent is disclosed in Bartollota et al. U.S. Patent 3,933,672. Other particularly

useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126 published April 28, 1977. An example of such a compound is DC-544, commercially availably from Dow Corning, which is a siloxane/glycol copolymer.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight. The incorporation of the suds modifiers is preferably made as separate particulates, and this permits the inclusion therein of other suds controlling materials such as C20-C24 fatty acids, microcrystalline waxes and high MW copolymers of ethylene oxide and propylene oxide which would otherwise adversely affect the dispersibility of the matrix. Techniques for forming such suds modifying particulates are disclosed in the previously mentioned Bartolotta et al U.S. Patent No. 3,933,672.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric polycarboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in the commonly assigned US Patent Nos. 4116885 and 4711730 and European Published Patent Application No. 0 272 033. A particular preferred polymer in accordance with EP-A-0 272 033 has the formula

$(CH_3(PEG)_{43})_{0.75}(POH)_{0.25}[T\text{-}PO)_{2.8}(T\text{-}PEG)_{0.4}]T(PO\text{-}H)_{0.25}((PEG)_{43}CH_3)_{0.75}$

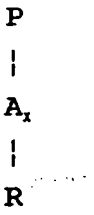
where PEG is $-(OC_2H_4)O-$, PO is (OC_3H_6O) and T is $(pcOC_6H_4CO)$.

Also very useful are modified polyesters as random copolymers of dimethyl terephtalate, dimethyl sulfoisophtalate, ethylene glycol and 1-2 propane diol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or propane-diol. The target is to obtain a polymer capped at both ends by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be end-capped by sulphobenzoate groups. However, some copolymers will be less than fully capped and therefore their end groups may consist of monoester of ethylene glycol and/or propane 1-2 diol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46 % by weight of dimethyl terephtalic acid, about 16 % by weight of propane -1.2 diol, about 10 % by weight ethylene glycol, about 13 % by weight of dimethyl sulfobenzoid acid and about 15 % by weight of sulfoisophtalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in EPA 311 342.

Certain polymeric materials such as polyvinyl pyrrolidones typically of MW 5000-20000, preferably 10000-15000, also form useful agents in preventing the transfer of labile dyestuffs between fabrics during the washing process. Especially preferred detergent ingredients are combinations with technologies which also provide a type of colour care benefit. Examples of these technologies are polyamide-N-oxide containing polymers such as disclosed in co-pending European Patent Application nr 92.202.168.6 (shortly disclosed hereunder).

These polymers contain units having the following structural formula I



(I)

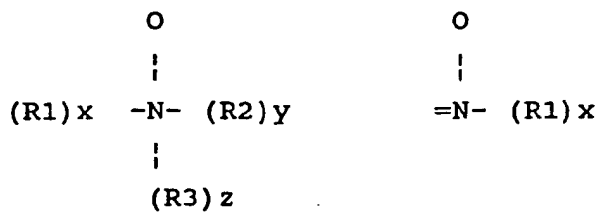
wherein P is a polymerizable unit, whereto the N-O group can be attached to or wherein the N-O group forms part of the polymerisable unit or a combination of both;

A is 

x is 0 or 1;

R are aliphatic, ethoxylated aliphatics, aromatic, heterocyclic or alicyclic groups or any combination thereof whereto the nitrogen of the N-O group can be attached or wherein the nitrogen of the N-O group is part of these groups.

The N-O group can be represented by the following general structures:



wherein R1, R2, and R3 are aliphatic groups, aromatic, heterocyclic or alicyclic groups or combinations thereof, x or/and y or/and z is 0 or 1 and wherein the nitrogen of the N-O group can be attached or wherein the nitrogen of the N-O group forms part of these groups.

The N-O group can be part of the polymerisable unit (P) or can be attached to the polymeric backbone or a combination of both.

Suitable polyamine N-oxides wherein the N-O group forms part of the polymerisable unit comprise polyamine N-oxides wherein R is selected from aliphatic, aromatic, alicyclic or heterocyclic groups. One class of said polyamine N-oxides comprises the group of polyamine N-oxides wherein the nitrogen of the N-O group forms part of the R-group. Preferred polyamine N-oxides are those wherein R is a heterocyclic group such as pyrridine, pyrrole, imidazole, pyrrolidine, piperidine, quinoline, acridine and derivatives thereof. Another class of said polyamine N-oxides comprises the group of polyamine N-oxides wherein the nitrogen of the N-O group is attached to the R-group.

Other suitable polyamine N-oxides are the polyamine oxides whereto the N-O group is attached to the polymerisable unit. Preferred class of these polyamine N-oxides are the polyamine N-oxides having the general formula (I) wherein R is an aromatic, heterocyclic or alicyclic groups wherein the nitrogen of the N-O functional group is part of said R group.
Examples of these classes are polyamine oxides wherein R is a heterocyclic compound such as pyrridine, pyrrole, imidazole and derivatives thereof.

Another preferred class of polyamine N-oxides are the polyamine oxides having the general formula (I) wherein R are aromatic, heterocyclic or alicyclic groups wherein the nitrogen of the N-O functional group is attached to said R groups.
Examples of these classes are polyamine oxides wherein R groups can be aromatic such as phenyl.

Any polymer backbone can be used as long as the amine oxide polymer formed is water-soluble and has dye transfer

inhibiting properties. Examples of suitable polymeric backbones are polyvinyls, polyalkylenes, polyesters, polyethers, polyamide, polyimides, polyacrylates and mixtures thereof.

The amine N-oxide polymers of the present invention typically have a ratio of amine to the amine N-oxide of 10:1 to 1:1000000. However the amount of amine oxide groups present in the polyamine N-oxide containing polymer can be varied by appropriate copolymerization or by appropriate degree of N-oxidation. Preferably, the ratio of amine to amine N-oxide is from 2:3 to 1:1000000. More preferably from 1:4 to 1:1000000, most preferably from 1:7 to 1:1000000. The polymers encompass random or block copolymers where one monomer type is an amine N-oxide and the other monomer type is either an amine N-oxide or not. The amine oxide unit of the polyamine N-oxides has a pKa < 10, preferably pKa < 7, more preferred pKa < 6.

The polyamine N-oxide containing polymer can be obtained in almost any degree of polymerisation. The degree of polymerisation is not critical provided the material has the desired water-solubility and dye-suspending power.

Typically, the average molecular weight of the polyamine N-oxide containing polymer is within the range of 500 to 1000,000; preferably from 1,000 to 50,000, more preferably from 2,000 to 30,000, most preferably from 3,000 to 20,000.

The polyamine N-oxide containing polymers are typically present from 0.001 to 10%, more preferably from 0.01 to 2%, most preferred from 0.05 to 1% by weight of the detergent composition.

Other colour-care technologies may be based on the use of peroxidases.

Fabric softening agents can also be incorporated into detergent compositions in accordance with the present invention.

These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1,400,898. Organic fabric softening agents include the water-insoluble tertiary amines as disclosed in GB-A-1514276 and EP-B-0 011 340 and their combination with mono C12-C14 quaternary ammonium salts are disclosed in EP-B-0 026 527 and EP-B-0 026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to 20%, more preferably from 8% to 15% by weight with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or di-long-chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water-soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as a molten liquid on to other solid components of the composition.

Enzymes other than the specific cellulase components comprised by the detergent compositions of the present invention can be present in the composition, such as proteases, lipases, esterases, peroxidases, oxidases, amylases and other classes of cellulases as well.

MAKING PROCESS

Compositions according to the present invention can be made via a variety of methods including dry mixing, spray drying, agglomeration and granulation and combinations of any of these techniques.

PREFERRED MAKING PROCESS

A preferred method of making the compositions herein involves a combination of spray drying, agglomeration in a high speed mixer and dry mixing.

A first granular component containing a relatively insoluble anionic surfactant is spray dried and part of the spray dried product is diverted and subjected to a low level of nonionic surfactant spray on before being reblended with the remainder. A second granular component is made by dry neutralisation of an anionic surfactant acid using sodium carbonate as the neutralising agent in a continuous high speed blender such as a Lodige KM mixer. The first and second components together with other dry mix ingredients such as the carboxylate chelating agent, inorganic peroxygen bleach, bleach activator, soil suspension agent, silicate and enzyme are then fed to a conveyor belt from which they are transferred to a horizontally rotating drum in which perfume and silicone suds suppressor are sprayed on to the product. In highly preferred compositions, a further drum mixing step is employed in which a low (approx. 2%) level of finely divided crystalline aluminosilicate is introduced to increase density and improve granular flow characteristics.

The present detergent compositions are in granular form and are characterized by their density, which is higher than the density of conventional detergent compositions. The density of the compositions herein ranges from 550 to 950g/liter, preferably 650 to 850 g/liter of composition, measured at 20°C.

The "compact" form of the compositions herein is best reflected, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; In conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition.

In the present compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition.

Inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides.

A preferred filler salt is sodium sulphate.

PROCESS OF WASHING

The compact detergent compositions herein have the ability to achieve the same efficiency than conventional detergent compositions, when a considerably lesser amount of composition herein, is used in the main wash cycle of a washing machine.

Accordingly, in an other embodiment of the invention, it is herewith provided for a process for washing fabrics in a washing machine wherein an amount of from 15 to 170 g of a detergent composition according to the present invention is used for the main wash cycle.
Typically, under European conditions, the recommended usage is from 80 to 140 g of detergent composition for the main wash cycle, without the need of a pre-wash.

The detergent compositions herein are preferably delivered directly to the drum and not indirectly via the outer casing of the machine. This can most easily be achieved by incorporation of the composition in a bag or container from which it can be released at the start of the wash cycle in response to agitation, a rise in temperature or immersion in the wash water in the drum. Such a container will be placed in the drum, together with the fabrics to be washed. Alternatively the washing machine itself may be adapted to permit direct addition of the composition to the drum e.g. by a dispensing arrangement in the access door.

Products comprising a detergent composition enclosed in a bag or container are usually designed in such a way that container integrity is maintained in the dry state to prevent egress of the contents when dry, but are adapted for release of the container contents on exposure to a washing environment, normally on immersion in an aqueous solution.

Usually the container will be flexible, such as a bag or pouch. The bag may be of fibrous construction coated with a water impermeable protective material so as to retain the contents, such as is disclosed in European published Patent Application No. 0 018 678. Alternatively it may be formed of a water insoluble synthetic polymeric material provided with an edge seal or closure designed to rupture in aqueous media as disclosed in European published Patent Application Nos. 0 011 500, 0 011 501, 0 011 502, and 0 011 968. A convenient form of water frangible closure comprises a water soluble adhesive disposed along and sealing one edge of a pouch formed of a water impermeable polymeric film such as polyethylene or polypropylene.

In a variant of the bag or container product form, laminated sheet products can be employed in which a central flexible layer is impregnated and/or coated with a composition and then one or more outer layers are applied to produce a fabric-like aesthetic effect. The layers may be sealed

together so as to remain attached during use or may separate on contact with water to facilitate the release of the coated or impregnated material.

An alternative laminate form comprises one layer embossed or deformed to provide a series of pouch-like containers into each of which the detergent components are deposited in measured amounts, with a second layer overlying the first layer and sealed thereto in those areas between the pouch-like containers where the two layers are in contact. The components may be deposited in particulate, paste or molten form and the laminate layers should prevent egress of the contents of the pouch-like containers prior to their addition to water. The layers may separate or may remain attached together on contact with water, the only requirement being that the structure should permit rapid release of the contents of the pouch-like containers into solution. The number of pouch-like containers per unit area of substrate is a matter of choice but will normally vary between 500 and 25,000 per square metre.

Suitable materials which can be used for the flexible laminate layers in this aspect of the invention include, among others, sponges, paper and woven and non-woven fabrics.

However the preferred means of carrying out the washing process according to the present invention includes the use of a reusable dispensing device having walls that are permeable to liquid but impermeable to the solid composition.

Devices of this kind are disclosed in European Patent Application Publication Nos. 0 343 069 and 0 344 070. The latter Application discloses a device comprising a flexible sheet in the form of a bag extending from a support ring defining an orifice, the orifice being adapted to admit to the bag sufficient product for one washing cycle in a washing cycle. A portion of the washing medium flows through the orifice into the bag, dissolves the product, and the solution then

passes outwardly through the orifice into the washing medium. The support ring is provided with a masking arrangement to prevent egress of wetted, undissolved, product, this arrangement typically comprising radially extending walls extending from a central boss in a spoked wheel configuration, or a similar structure in which the walls have a helical form.

METHODS

DETERMINATION OF ACTIVITY TOWARDS LABELLED MICROCRYSTALLINE CELLULOSE

PREPARATION OF RED AVICEL SUBSTRATE

The Red Avicel substrate was prepared as follows:

Avicel® is a microcrystalline cellulose product which is manufactured by Asahi Chemical Co. Ltd., Japan. 162 g of Avicel® corresponds to 1 mole of the glucose units forming the cellulose polymeric chains of Avicel.

As the reactive dye was used the dye Procion® Red H-E3B which is manufactured by Imperial Chemical Industries Ltd., (ICI), U.K.

The reactive dye was covalently bound to Avicel® in accordance with the directions for use with cotton which were provided by the dye manufacturer.

A solution of 10 g/l of Procion® Red H-E3B in distilled water was prepared and stirred overnight at 20°C. The solution was centrifuged at 5000 rpm for 20 min. and the sediment was removed.

10 g of Avicel® was placed in a 250 ml conic flask. 50 ml of the dye solution was added and the mixture was shaken at

room temperature for 1 h. The mixture was slowly heated to 50°C for 30 min., followed by addition of 1 ml of Na_2SO_4 suspension in hot water (500 g/l anhydrous Na_2SO_4).

The mixture was slowly heated to 90°C for approx. 45 min. During this heating period 3 ml of Na_2SO_4 in hot water (500 g/l anhydrous Na_2SO_4) was added to the mixture after approx. 15 min. and additionally 6 ml of Na_2SO_4 in hot water (500 g/l anhydrous Na_2SO_4) was added after approx. 30 min.

The mixture was allowed to stand for 20 min at 85°C. Then 3x1 ml of alkaline solution (100 g/l Na_2CO_3, 4 g/l NaOH) was added at 5 min. intervals. The resulting mixture was shaken at 85°C for 1 h and was allowed to cool overnight.

The mixture was centrifuged at 4000 rpm at 25°C for 15 min. The supernatant was removed and 60 ml of water was added to the sediment. The mixture was stirred for 30 min. on a magnetic stirrer, followed by centrifugation for 15 min. This procedure was repeated until the supernatant was no longer coloured, and the resulting sediment was lyophilized to yield a dry dyed substrate, Red Avicel.

METHOD OF MEASUREMENT
CATALYTIC ACTIVITY ON RED AVICEL

A substrate suspension containing 40 g/l of Red Avicel prepared as described above (corresponding to 5 g of dye per 162 g of dry Avicel®) in 0.1 M Tris-HCl buffer, pH 7.5, was prepared.

The enzyme sample to be determined was dissolved in the same buffer.

0.5 ml of substrate suspension and 0.5 ml of enzyme solution were mixed and mounted in a microbiological shaker thermostated at 40°C. After 2 h the reaction was stopped by centrifuging the mixture at 4000 g at 4°C. The supernatant was

transferred to a narrow 1 cm cuvette and the absorbance was measured at a wavelength of 536 nm.

Calculations and resulting definition:

The total dye load of Red Avicel prepared as described above was estimated by monitoring the absorbance at a wave length of 536 nm of a solution of the dyed substrate in 85% phosphoric acid. Correction was made for the difference in absorbance measured in the phosphoric acid and the buffer, respectively.

This correction was determined from the comparison of the monitored absorbance at 536 nm of the (unbound) red dye in 85% phosphoric acid and Tris-HCl buffer, respectively:

ABSORBANCE (536 nm)	85% phosphoric acid	0.1 M Tris-HCl buffer
0.1 g/l unbound red dye	0.88 O.D.	0.93 O.D.
Red Avicel	44 O.D.	46.5 O.D.*

*: calculated value [44 O.D.x(0.93/0.88)].
O.D.: Optical Density

The concentration of coloured product released from the substrate may be calculated from the total dye load per 1 mole of glucose units in the substrate (Red Avicel), i.e. 5 g of red dye per 162 g of dry Avicel®, under the assumption that the dyeing process did proceed uniformly along the profile of susceptance to enzymatic hydrolysis.

The measured optical density (O.D.) minus corresponding blank was plotted versus the enzyme concentration (mg enzyme protein/ml). The initial region of the curve up to 0.2 O.D. above blank was used for calculations.

Accordingly, 1 IU of enzyme activity towards Red Avicel, i.e. Avicel® dyed with Procion® Red H-E3B, is defined as the amount of enzyme capable of solubilising 1 mmole/min. of coloured product as glucose units corresponding to 0.046 O.D./min. of Red Avicel in a total volume of 1 litre.

DETERMINATION OF CELLULASE ACTIVITY (S-CEVU)

The cellulase enzymes hydrolyse CMC, thereby increasing the viscosity of the incubation mixture.

Determination of the cellulase activity, measured in terms of S-CEVU, was determined according to the analysis method AF 302/2-GB which is available from the Applicant upon request.

The S-CEVU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxymethylcellulose (CMC). The assay is carried out at 40°C, pH 7.5 using a relative enzyme standard for reducing the viscosity of the CMC substrate.

CELLULASE ACTIVITY ON CELLOTRIOSE

The cellulase activity on cellotriose, in terms of k_{cat} (s^{-1}), was determined by a coupled assay:

Cellotriose → Glucose + Cellobiose (cat.: cellulase)

Glucose + O_2 + H_2O → Gluconate + H_2O_2 (cat.: Glucoseoxidase)

H_2O_2 + $ABTS^R$ → $ABTS^{Ox}$ (cat.: Peroxidase)

which is followed spectrophotometrically at 418 nm (maximum absorbance of $ABTS^{Ox}$ at 418 nm).

Method:

The GOD-Perid Test Kit (available from Boehringer Mannheim, art. 124 036) was used. The buffer-enzyme solution in the test kit was dissolved in 500 ml milli Q water. pH of the solution was adjusted to 8.5 (NaOH).

80 mg of $ABTS^{R}$ (available from Boehringer Mannheim, art. 756 407) was dissolved in 10 ml GOD-Perid corresponding to a total concentration of $ABTS^{R}$ of 10 mg/ml.

A substrate stock solution of 5 mmole (2.52 mg/ml) of cellotriose (available from Merck art. 24741) in water was prepared. Diluted solutions in water corresponding to 1000 µmole, 500 µmole, 376 µmole, 250 µmole, 100µmole and 60 µmole were prepared.

The reaction mixture was prepared by mixing 1 part of substrate solution with 1 part of GOD-Perid.

A solution of the cellulase enzyme to be determined in a concentration of 1.0 - 3.0 µmole was prepared.

50 µl of enzyme solution and 450 µl of reaction mixture were mixed.

The measurements were carried out on a HP 8452A Diode Array Spectrophotometer thermostated at 40°C, 1 cm cuvette, at a wavelength of 418 nm. The reaction was followed by measuring the oxidation of ABTS every 20 sec for 600 sec in total.

Calculations:

The cellulase activity on cellotriose, in terms of k_{cat} (s^{-1}), was calculated from a Lineweaver-Burk plot (a plot of 1/V

versus 1/[S]): the slope and the intersection were determined by linear regression analysis.

The following constants were used for the calculations:

Cellulase: ϵ = 66,310 $M^{-1} \cdot cm^{-1}$

$ABTS^{Ox}$: ϵ = 0.0323 $\mu mole^{-1} \cdot cm^{-1}$

The following examples illustrate the invention and facilitate its understanding.

EXAMPLE 1

Determination of cellulase activity (measured in S-CEVU), activity towards cellotriose and activity towards dyed microcrystalline cellulose, respectively, was carried out as described above.

These determinations were carried out for the enzymes, i.e. the cellulase components, listed in the following TABLE I together with the determined activities. CBH I, EG I and EG I-F have retaining-type activity (*Eur. J. Biochem., 217, p. 947-953 (1993)*).

TABLE I

ENZYME	Molecular weight (kD)	Activity per mg protein		Act. on cello-triose $k_{cat}(s^{-1})$
		S-CEVU*	Red Avicel Units	
First cellulase components:				
CBH I	70	0	0.0000242	0.015
EG I	50	200	0.0000354	1.5
EG I-F	50	465	0.0000252	5.5
Second cellulase components:				
EG II	50	200	0.00021	0
EG III	26	14	n.a.	0
EG V	43	430	0.002204	0
EG V core	22	700	0.002043	0
EG VI	38	150	0.000424	0
EG C	60	n.a.	0.002511	0

*: S - CELLULASE VISCOSITY UNIT

The results show that the cellulase components denoted CBH I, EG I and EG I-F have a very low catalytic activity on Red Avicel as compared to the cellulase components EG II, EG III, EG V EG V core, EG VI and EG C, which all exhibit a catalytic activity on Red Avicel at pH 7.5 per 1 mg of cellulase protein corresponding to an adsorption higher than 10^{-4} at a wavelength of 536 nm. Accordingly, the cellulase components EG II, EG III, EG V EG V core, EG VI and EG C are

capable and effective of colour clarification when used for washing cellulose-containing fabrics. The mentioned cellulase components are also capable of particulate soil removal but their capability of particulate soil removal is combined with a moderate fabric damage which is in contrast to the particulate soil removal capability of the cellulase components CBH I and EG I, see below.

Furthermore, it is shown that the cellulase components CBH I, EG I and EG I-F exhibit a catalytic activity on cellotriose at pH 8.5, whereas EG II, EG III, EG V EG V core, EG VI and EG C do not exhibit any activity on cellotriose. Accordingly, the cellulase components CBH I, EG I-F and EG I, when used in a dosage range of 0.001 - 100 mg are capable of performing particulate soil removal without damaging the fabric and without performing colour clarification.

EXAMPLE II

A. Stain Removal

Test Procedure

4 carbon black stained swatches (5 x 7.5 cm) were washed in a Linitest with 10 stainless steel balls for agitation, at 40°C. The detergent concentration was 0.7%, tap water was used. Each Linitest pot was filled with 400 ml detergent solution. The wash cycle time was 60 minutes. After each cycle the swatches were rinsed, each swatch separately, under tap water. All the swatches were then rinsed together and rinsed in a washing machine.

The reference detergent is a European-type with comprising no enzymes, and no dye transfer inhibitor polymer + citric acid to pH 7.

Stain removal vs. an unwashed carbon black stained swatch was measured by spectrophotometric reflectance using a Spectraflash 500 after 2 wash cycles. Percentage stain removal was expressed as the percentage difference in reflectance versus the unwashed swatch. The result of the measurements is shown in the table below. The figures are mean values of 4 carbon black stained swatches.

	% Stain Removal
Reference (Ref)	16
Ref + EG I (100 S-CEVU/400 ml)	32
Ref + EG V (100 S-CEVU/400 ml)	17
Ref + EG I (100 S-CEVU/400 ml) + EG V (100 S-CEVU/400 ml)	35

B. Depilling/Colour Clarification

Test Procedure

4 blue underwear swatches (old pyjamas fabric, size 10 x 7.5 cm) were washed in a Linitest, with 10 stainless steel balls for agitation, at 40°C. The detergent concentration was 0.7%, tap water was used. Each Linitest pot was filled with 400 ml detergent solution. The wash cycle time was 60 minutes. After each cycle the underwear swatches were rinsed, each swatch separately, under tap water. All the swatches were then rinsed together in a washing machine.

The reference detergent is a European-type detergent composition with no enzymes and no dye transfer inhibitor polymer + citric acid to pH 7.

Visual grading (*) vs. the reference (without enzymes) was performed after 5 wash cycles. The result of the measure-

ments is shown in the table below. The figures are mean values of 4 underwear swatches.

* 0 = Equally good
1 = Slightly better
3 = Much better
4 = Excellent

Blue underwear	Depilling	
EG I (100 S-CEVU/400ml)	EG V (100 S-CEVU/400ml)	EG I (100 S-CEVU/400ml) + EG V (100 S-CEVU/400ml)
-0.09	1.63 (s0.33)	2.84 (s0.33)

EXAMPLES III to XIX

The following compositions are made wherein or to which the first and second cellulase components may be present or added.

a) Compact granular detergent : examples III and IV.

Example	III	IV
Tallow alkyl sulphate	1.80	2.40
C_{45} alkyl sulphate	14.00	13.10
C_{45} alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00

Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta(methylene phosphonic acid) (DETMPA)	1.00	0.20
Protease (4 KNPU)	0.60	0.60
Lipase (100 KLU)	0.36	0.40
Amylase (60 KNU)	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
PVP	0.30	0.50
Minors	up to 100	

b) conventional granular detergent : examples V and VI

Example	V	VI
Alkyl sulphate	6.5	8.0
Sodium sulphate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
PVP	0.5	0.7
TAED	3.0	3.0
Perborate	15.0	-
Minors	up to 100	

c) liquid detergent : examples VII and VIII

The liquid detergent compositions of the present invention comprise an effective amount of the first and second cellulase component, preferably from 0.0001% to 10%, more preferably from 0.001% to 1% and most preferably from 0.001% to 0.1% by weight of cellulase enzyme protein in the composition.

Example	VII	VIII
C_{12-14} alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C_{12-15} alkyl sulphate	8.0	8.0
Sodium sulphate of C12-15 alcohol 2 times ethoxylated	-	3.0
C_{12-15} alcohol 7 times ethoxylated	-	8.0
C_{12-15} alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta(methylene phosphonic acid) (DETMPA)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Protease (4 KNPU)	0.2	0.2
PVP	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Waters and minors	up to 100 parts	

d) granular detergent compositions: examples IX - XIII

The granular detergent compositions of the present invention contain an effective amount of the first and second cellulase component, preferably from 0.001% to 10%, more preferably from 0.005% to 5%, and most preferably from 0.01% to 1% by weight of total cellulase enzyme protein in the composition.

Example	IX	X	XI	XII	XIII
Alkyl sulphate	8.0	20.0	7	4.5	-
Alkyl ethoxysulphate	2.0	6.0	5	5.5	9.5
Mixture of C_{25} and C_{45} alcohol 3 and 7times ethoxylated	6.0	3.0	5	-	-
Polyhydroxy fatty acid amide	2.5	-	-	-	-
Linear alkylbenzene sulphonate	-	-	-	4.0	10.0

Zeolite	17.0	20.0	10.0	4.0	0.3
Layered silicate/citrate	16.0	12.5	10.0	4.0	0.3
Carbonate	7.0	23.0	5.0	10.0	24.0
Nonanoyl Caprolactam	-	-	5.0	-	-
Maleic acid acrylic acid copolymer	5.0	-	4.0	5.0	5.0
Soil release polymer	0.4	-	0.2	-	-
Protease (4 KNPU)	2.5	1.5	0.3	1.0	1.5
Lipase (100 KLU)	0.2	-	0.3	0.2	0.2
Perborate	-	3.0	-	22.0	-
TAED	6.0	-	-	6.0	-
Percarbonate	22.0	-	15.0	-	-
EDDS	0.3	-	0.4	-	-
Suds suppressor	3.5	0.32	2.0	0.7	1.5
Water, perfume and minors	up to 100 parts				

e) liquid detergent compositions: examples XIV - XVII

Examples	XIV	XV	XVI	XVII
C_{12}-C_{14} alkyl sulphate (sodium)	20.0	12.0	10.0	11.5
2-Butyl octanoic acid	5.0	7.0	-	-
Sodium citrate	1.0	2.5	-	3.0
C_{10} alcohol ethoxylate (3)	13.0	3.5	25.0	9.5
Monoethanol amine	2.5	6.0	-	-
Fatty acid	-	10.0	14.0	0.1
Propane diol	8.0	15.0	8.0	4.5
Lipase (100 KLU)	-	0.15	-	0.9
Amylase (66 KNU)	-	0.10	-	-
Protease (4 KNPU)	-	0.50	1.2	0.5
Soil release agent	-	0.50	-	-
Water/propylene glycol/ethanol	up to 100 parts			

f) bar fabric cleaning compositions

A laundry bar suitable for hand-washing soiled fabrics is prepared by standard extrusion processes. The bars contain an effective amount of the first and second cellulase component, preferably from 0.001% to 10%, more preferably from 0.01% to 1% by weight of the composition and comprises the following:

Example XVIII

Component	Weight %
Alkyl sulphate	30
Phosphate (as sodium tripolyphosphate)	7
Sodium carbonate	25
Sodium pyrophosphate	7
Coconut monoethanolamide	2
Zeolite A (0.1-10 micron)	5
Carboxymethylcellulose	0.2
Polyacrylate (m.w. 1400)	0.2
(6-Nonanamidocaproyl)oxybenzenesulfonate	5
Sodium Percarbonate	5
Brightener, perfume	0.2
Protease	0.3**
Lipase (100 KNU)	0.3
$CaSO_4$	1
$MgSO_4$	1
Water	4
Filler*	Balance to 100%

* Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

** Denotes mg of active enzyme per gram of composition.

The detergent laundry bars are processed in conventional soap or detergent bar making equipment as commonly used in the art.

EXAMPLE XIX

Compact granular detergent:

	w/w%
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C25 and C45 alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
CMC	0.4
Poly (4-vinylpyridine)-N-oxide	0.1
PEG2000	0.2
Protease (4 KNPU)	2.5
Lipase (100 KLU)	0.2
EG V (1000 S-CEVU)	0.2
EG I (1250 S-CEVU)	1.0
TAED	6.0
Percarbonate	22.0
Ethylene Diamine Disuccinic acid (EDDS)	0.3
Suds suppressor	3.5
Disodium-4.4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyril) biphenyl	0.05
Water, Perfume (Encaps) and Minors	up to 100 parts

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsvaerd
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): DK-2880
(G) TELEPHONE: +45 4444 8888
(H) TELEFAX: +45 4449 3256
(I) TELEX: 37173

(A) NAME: The Procter & Gamble Company
(B) STREET: One Procter & Gamble Plaza
(C) CITY: Cincinnati
(D) STATE: OHIO
(E) COUNTRY: U.S.A.
(F) POSTAL CODE (ZIP): 45202

(ii) TITLE OF INVENTION: A detergent composition comprising cellulolytic enzymes

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola griseus

(x) PUBLICATION INFORMATION:
(A) AUTHORS: de Oliviera Azevedo, M.
Radford, A.
(C) JOURNAL: Nucleic Acids Res.
(D) VOLUME: 18
(F) PAGE: 668
(G) DATE: 1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gln Gln Ala Cys Ser Leu Thr Thr Glu Arg His Pro Ser Leu Ser Trp
1 5 10 15

Asn Lys Cys Thr Ala Gly Cys Gln Cys Gln Thr Val Gln Ala Ser Ile
20 25 30

Thr Leu Asp Ser Asn Trp Arg Trp Thr His Gln Val Ser Gly Ser Thr
35 40 45

Asn Cys Tyr Thr Gly Asn Lys Trp Asp Thr Ser Ile Cys Thr Asp Ala
50 55 60

Lys Ser Cys Ala His Asn Cys Cys Val Asp Gly Ala Tyr Thr Ser Thr
65 70 75 80

Tyr Gly Ile Thr Thr Asn Gly Asp Ser Leu Ser Ser Leu Lys Phe Val
85 90 95

Thr Lys Gly Gln His Ser Thr Asn Val Gly Ser His Thr Tyr Leu Met
100 105 110

Asp Gly Glu Asp Lys Tyr Gln Thr Phe Glu Leu Leu Gly Asn Glu Phe
115 120 125

Thr Thr Asp Val Asp Val Ser Asn Ile Gly Cys Gly Leu Asn Gly Ala
130 135 140

Thr Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Leu Ser Arg Tyr Pro
145 150 155 160

Cys Asn Lys Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ala Gln
165 170 175

Cys Pro Arg Asp Ile Lys Phe Ile Asn Gly Glu Ala Asn Ile Glu Gly
180 185 190

Trp Thr Gly Ser Thr Asn Asp Pro Asn Ala Gly Ala Cys Ser Arg Tyr
195 200 205

Gly Thr Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Gln Gln His Ala
210 215 220

Thr Ala Phe Pro His Pro Cys Thr Ile Ile Ala Gln Ser Arg Cys Glu
225 230 235 240

Gly Asp Ser Cys Gly Gly Thr Tyr Ser Asn Glu Arg Tyr Ala Gly Val
245 250 255

Cys Asp Pro Asp Gly Cys Asp Phe Asn Ser Tyr Arg Gln Gly Asn Lys
260 265 270

Thr Phe Tyr Gly Lys Gly Met Thr Val His Thr Thr Lys Lys Ile Thr
275 280 285

Val Val Thr Pro Phe Leu Lys Asp Ala Asn Gly Asp Leu Gly Glu Ile
290 295 300

Lys Arg Phe Tyr Val Gln Asp Gly Lys Ile Ile Pro Asn Ser Glu Ser
305 310 315 320

Thr Ile Pro Gly Val Glu Gly Asn Ser Ile Thr Gln Asp Trp Cys Asp
325 330 335

Arg Gln Lys Val Ala Phe Gly Asp Ile Asp Asp Phe Asn Arg Lys Gly
340 345 350

Gly Ala Met Lys Gln Met Gly Lys Ala Leu Ala Gly Pro Met Val Leu
355 360 365

Met Ser Ile Trp Asp Asp His Ala Ser Asn Met Leu Trp Leu Asp Ser
370 375 380

Thr Phe Pro Val Asp Ala Ala Gly Lys Pro Gly Ala Glu Arg Gly Ala
385 390 395 400

Cys Pro Thr Thr Ser Gly Val Pro Ala Glu Val Glu Ala Glu Ala Pro
405 410 415

Asn Ser Asn Val Val Phe Ser Asn Ile Arg Pro Gly Pro Ile Gly Ser
420 425 430

Thr Val Ala Gly Leu Pro Gly Ala Gly Asn Gly Gly Asn Asn Gly Gly
435 440 445

Asn Pro Pro Pro Pro Thr Thr Thr Thr Ser Ser Ala Pro Ala Thr Thr
450 455 460

Thr Thr Ala Ser Ala Gly Pro Lys Ala Gly Arg Trp Gln Gln Cys Gly
465 470 475 480

Gly Ile Gly Phe Thr Gly Pro Thr Gln Cys Glu Glu Pro Tyr Ile Cys
485 490 495

Thr Lys Leu Asn Asp Trp Tyr Ser Gln Cys Leu
500 505

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola insolens
(B) STRAIN: DSM 1800

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Lys Pro Gly Glu Thr Lys Glu Val His Pro Gln Leu Thr Thr Phe
1 5 10 15

Arg Cys Thr Lys Arg Gly Gly Cys Lys Pro Ala Thr Asn Phe Ile Val
20 25 30

Leu Asp Ser Leu Ser His Pro Ile His Arg Ala Glu Gly Leu Gly Pro
35 40 45

Gly Gly Cys Gly Asp Trp Gly Asn Pro Pro Pro Lys Asp Val Cys Pro
50 55 60

Asp Val Glu Ser Cys Ala Lys Asn Cys Ile Met Glu Gly Ile Pro Asp
65 70 75 80

Tyr Ser Gln Tyr Gly Val Thr Thr Asn Gly Thr Ser Leu Arg Leu Gln
85 90 95

His Ile Leu Pro Asp Gly Arg Val Pro Ser Pro Arg Val Tyr Leu Leu
100 105 110

Asp Lys Thr Lys Arg Arg Tyr Glu Met Leu His Leu Thr Gly Phe Glu
115 120 125

Phe Thr Phe Asp Val Asp Ala Thr Lys Leu Pro Cys Gly Met Asn Ser
130 135 140

Ala Leu Tyr Leu Ser Glu Met His Pro Thr Gly Ala Lys Ser Lys Tyr
145 150 155 160

Asn Pro Gly Gly Ala Tyr Tyr Gly Thr Gly Tyr Cys Asp Ala Gln Cys
165 170 175

Phe Val Thr Pro Phe Ile Asn Gly Leu Gly Asn Ile Glu Gly Lys Gly
180 185 190

Ser Cys Cys Asn Glu Met Asp Ile Trp Glu Ala Asn Ser Arg Ala Ser
195 200 205

His Val Ala Pro His Thr Cys Asn Lys Lys Gly Leu Tyr Leu Cys Glu
210 215 220

Gly Glu Glu Cys Ala Phe Glu Gly Val Cys Asp Lys Asn Gly Cys Gly
225 230 235 240

Trp Asn Asn Tyr Arg Val Asn Val Thr Asp Tyr Tyr Gly Arg Gly Glu
245 250 255

Glu Phe Lys Val Asn Thr Leu Lys Pro Phe Thr Val Val Thr Gln Phe
260 265 270

Leu Ala Asn Arg Arg Gly Lys Leu Glu Lys Ile His Arg Phe Tyr Val
275 280 285

```
Gln Asp Gly Lys Val Ile Glu Ser Phe Tyr Thr Asn Lys Glu Gly Val
    290                 295                 300

Pro Tyr Thr Asn Met Ile Asp Asp Glu Phe Cys Glu Ala Thr Gly Ser
305                 310                 315                 320

Arg Lys Tyr Met Glu Leu Gly Ala Thr Gln Gly Met Gly Glu Ala Leu
                325                 330                 335

Thr Arg Gly Met Val Leu Ala Met Ser Ile Trp Trp Asp Gln Gly Gly
            340                 345                 350

Asn Met Glu Trp Leu Asp His Gly Glu Ala Gly Pro Cys Ala Lys Gly
        355                 360                 365

Glu Gly Ala Pro Ser Asn Ile Val Gln Val Glu Pro Phe Pro Glu Val
    370                 375                 380

Thr Tyr Thr Asn Leu Arg Trp Gly Glu Ile Gly Ser Thr Tyr Gln Glu
385                 390                 395                 400

Val Gln Lys Pro Lys Pro Lys Pro Gly His Gly Pro Arg Ser Asp
                405                 410                 415
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 409 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Fusarium oxysporum
- (B) STRAIN: DSM 2672

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Gln Thr Pro Asp Lys Ala Lys Glu Gln His Pro Lys Leu Glu Thr Tyr
1               5                   10                  15
```

Arg Cys Thr Lys Ala Ser Gly Cys Lys Lys Gln Thr Asn Tyr Ile Val
20 25 30

Ala Asp Ala Gly Ile His Gly Ile Arg Arg Ser Ala Gly Cys Gly Asp
35 40 45

Trp Gly Gln Lys Pro Asn Ala Thr Ala Cys Pro Asp Glu Ala Ser Cys
50 55 60

Ala Lys Asn Cys Ile Leu Ser Gly Met Asp Ser Asn Ala Tyr Lys Asn
65 70 75 80

Ala Gly Ile Thr Thr Ser Gly Asn Lys Leu Arg Leu Gln Gln Leu Ile
85 90 95

Asn Asn Gln Leu Val Ser Pro Arg Val Tyr Leu Leu Glu Glu Asn Lys
100 105 110

Lys Lys Tyr Glu Met Leu His Leu Thr Gly Thr Glu Phe Ser Phe Asp
115 120 125

Val Glu Met Glu Lys Leu Pro Cys Gly Met Asn Gly Ala Leu Tyr Leu
130 135 140

Ser Glu Met Pro Gln Asp Gly Gly Lys Ser Thr Ser Arg Asn Ser Lys
145 150 155 160

Ala Gly Ala Tyr Tyr Gly Ala Gly Tyr Cys Asp Ala Gln Cys Tyr Val
165 170 175

Thr Pro Phe Ile Asn Gly Val Gly Asn Ile Lys Gly Gln Gly Val Cys
180 185 190

Cys Asn Glu Leu Asp Ile Trp Glu Ala Asn Ser Arg Ala Thr His Ile
195 200 205

Ala Pro His Pro Cys Ser Lys Pro Gly Leu Tyr Gly Cys Thr Gly Asp
210 215 220

Glu Cys Gly Ser Ser Gly Ile Cys Asp Lys Ala Gly Cys Gly Trp Asn
225 230 235 240

His Asn Arg Ile Asn Val Thr Asp Phe Tyr Gly Arg Gly Lys Gln Tyr
245 250 255

Lys Val Asp Ser Thr Arg Lys Phe Thr Val Thr Ser Gln Phe Val Ala
260 265 270

Asn Lys Gln Gly Asp Leu Ile Glu Leu His Arg His Tyr Ile Gln Asp
275 280 285

Asn Lys Val Ile Glu Ser Ala Val Val Asn Ile Ser Gly Pro Pro Lys
290 295 300

Ile Asn Phe Ile Asn Asp Lys Tyr Cys Ala Ala Thr Gly Ala Asn Glu
305 310 315 320

Tyr Met Arg Leu Gly Gly Thr Lys Gln Met Gly Asp Ala Met Ser Arg
325 330 335

Gly Met Val Leu Ala Met Ser Val Trp Trp Ser Glu Gly Asp Phe Met
340 345 350

Ala Trp Leu Asp Gln Gly Val Ala Gly Pro Cys Asp Ala Thr Glu Gly
355 360 365

Asp Pro Lys Asn Ile Val Lys Val Gln Pro Asn Pro Glu Val Thr Phe
370 375 380

Ser Asn Ile Arg Ile Gly Glu Ile Gly Ser Thr Ser Ser Val Lys Ala
385 390 395 400

Pro Ala Tyr Pro Gly Pro His Arg Leu
405

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola insolens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro
-21 -20                 -15                 -10

Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys
 -5                   1                   5                  10

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
             15                  20                  25

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
         30                  35                  40

Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
     45                  50                  55

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
 60                  65                  70                  75

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
                 80                  85                  90

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
             95                  100                 105

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
        110                 115                 120

Ile Pro Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
    125                 130                 135

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
140                 145                 150                 155

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
                160                 165                 170

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
            175                 180                 185

Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
        190                 195                 200
```

Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser
205 210 215

Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr Ser Thr Thr
220 225 230 235

Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
240 245 250

Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys
255 260 265

Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys
270 275 280

Leu

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 724 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola insolens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCTAGGTCGC CCACCATGCG CGTTTCTCTT GCTCTCCTCG CCTACCTGCT CAGCGCCGCC 60

CCGGCCTCGC CCGTCCCGGA GCTCGAGCCC CGGCAGTCCG GCAACCCCTT CTCCGGCCGC 120

ACCCTGCTGG TCAACTCGGA CTATAGCAGC AAGCTCGACC AGACGCGCCA GGCCTTTCCT 180

GTCCCGCGGC GACCAGACCA ACGCTGCCAA GGTCAAGTAC GTCCAGGAGA AGGTTGGCAC 240

CTTTCTATTG GACTTCCAAC ATCTTCCTCC TGCGCAGCAC TGACGTTGCC ATCCAGAATG 300

CGCGCCGCCA AGGCCGCGCG AGAACCCCAT CGTCGGTCTC GTCCTGTACA ACCTCCCCGA 360

CCGCGACTGC AGCGACGCGG CAGTACCTCT GGCGACGTTA AGCTCTCCCA GAACGGCCTG 420

AACCGGTACA AGAACGAGTA CGTCAACCCG TTCGCCCAGA AGCTCAAGGC CGCGTCCGAC 480

GTGCAGTTCG CCGTCATCCT CGAGCCCGAT GCCATCGGCA ACATGGTCAC GGGCACCAGC 540

GCCTTCTGCC GCAACGCCCG CGGCCCTCAG AGGAGGCCAT CGGCTATGCT ATCTCTCCTC 600

GGCTGGGCCG ATAAGCTCGA GCCAACTGCC CAGGAGGTGC CACCATCCTC CAAAAGGCCG 660

GTAACAACGC AAGATCGCGG CTTCTCAGCA ACGTTCCAAC TACAACCTAT TCACGACAAC 720

CGCG
724

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 526 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus lautus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Arg Ile His Ala Ile Arg Gln Ser Cys Arg Leu Val Leu Thr Met
1 5 10 15

Val Leu Met Leu Gly Leu Leu Leu Pro Val Gly Ala Pro Lys Gly Tyr
20 25 30

Ala Ala Pro Ala Val Pro Phe Gly Gln Leu Lys Val Gln Gly Asn Gln
35 40 45

Leu Val Gly Gln Ser Gly Gln Ala Val Gln Leu Val Gly Met Ser Ser
50 55 60

His Gly Leu Gln Trp Tyr Gly Asn Phe Val Asn Lys Ser Ser Leu Gln
65 70 75 80

```
Trp Met Arg Asp Asn Trp Gly Ile Asn Val Phe Arg Ala Ala Met Tyr
                  85                  90                  95

Thr Ser Glu Asp Gly Tyr Ile Thr Asp Pro Ser Val Lys Asn Lys Val
            100                 105                 110

Lys Glu Ala Val Gln Ala Ser Ile Asp Leu Ala Leu Tyr Val Ile Ile
        115                 120                 125

Asp Trp His Ile Leu Ser Asp Gly Asn Pro Asn Thr Tyr Lys Ala Gln
    130                 135                 140

Ser Lys Ala Phe Phe Gln Glu Met Ala Thr Leu Tyr Gly Asn Thr Pro
145                 150                 155                 160

Asn Val Ile Tyr Glu Ile Ala Thr Ser Pro Thr Glu Cys Val Leu Gly
                165                 170                 175

Arg Cys Gln Ser Ser Glu Glu Val Ile Thr Ala Ile Arg Ser Ile Asp
            180                 185                 190

Pro Asp Gly Val Val Ile Val Gly Ser Pro Thr Trp Ser Gln Asp Ile
        195                 200                 205

His Leu Ala Ala Asp Asn Pro Val Ser His Ser Asn Val Met Tyr Ala
    210                 215                 220

Leu His Phe Tyr Ser Gly Thr His Gly Gln Phe Leu Arg Asp Arg Ile
225                 230                 235                 240

Thr Tyr Ala Met Asn Lys Gly Ala Ala Ile Phe Val Thr Glu Trp Gly
                245                 250                 255

Thr Ser Asp Ala Ser Gly Asn Gly Gly Pro Tyr Leu Pro Gln Ser Lys
            260                 265                 270

Glu Trp Ile Asp Phe Leu Asn Ala Arg Lys Ile Ser Trp Val Asn Trp
        275                 280                 285

Ser Leu Ala Asp Lys Val Glu Thr Ser Ala Ala Leu Met Pro Gly Ala
    290                 295                 300

Ser Pro Thr Gly Ala Gly Pro Met Pro Asn Cys Arg Met Gly Lys Ser
305                 310                 315                 320
```

Gly Ser Arg Ser Asn Pro Ala Ser Asn Trp Arg Arg Gln Gly Asn Pro
325 330 335

Thr Ala Pro Ala Ala Pro Thr Asn Leu Ser Ala Asn Gly Gly Asn Ala
340 345 350

Gln Val Ser Leu Thr Trp Asn Ala Val Ser Gly Ala Thr Ser Tyr Thr
355 360 365

Val Lys Arg Ala Thr Thr Ser Gly Gly Pro Tyr Thr Asn Val Asp Arg
370 375 380

Gly Val Thr Ala Thr Ser Tyr Thr Asn Thr Gly Leu Thr Asn Gly Thr
385 390 395 400

Thr Tyr Tyr Tyr Val Val Arg Ala Ser Asn Ser Ala Gly Ser Ser Ala
405 410 415

Asn Ser Ala Gln Ala Ser Ala Thr Pro Ala Ser Gly Gly Ala Ser Thr
420 425 430

Gly Asn Leu Val Val Gln Tyr Lys Val Gly Asp Thr Ser Ala Thr Asp
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Asn Gln Met Lys Pro Ser Phe Asn Ile Lys Asn Asn Gly Thr Thr Pro
450 455 460

Val Asn Leu Ser Gly Leu Lys Leu Xaa Xaa Xaa Xaa Xaa Lys Asp Gly
465 470 475 480

Pro Ala Asp Met Ser Cys Ser Ile Asp Trp Ala Gln Ile Gly Arg Thr
485 490 495

Asn Val Leu Leu Ala Phe Ala Asn Phe Thr Gly Ser Asn Thr Asp Thr
500 505 510

Tyr Cys Cys Glu Leu Ser Phe Ser Cys Thr Ala Gly Ser Tyr Pro Gly
515 520 525

Tyr Ala Trp
530

CLAIMS

1. Detergent composition comprising a first cellulase component having retaining-type activity and being capable of particulate soil removal and a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and being capable of colour clarification wherein at least one of the cellulase components is a single (recombinant) component.

2. A detergent composition according to claim 1 wherein the single (recombinant) component is present in an amount of at least 5%, based on the total weight of cellulase protein in the composition.

3. A detergent composition according to claim 1 or 2 wherein the first and the second component are single components.

4. A detergent composition according to any of the claims 1-3, wherein the first and the second cellulase component, respectively, is present in a concentration corresponding to a concentration in the washing liquor of 0.001 - 100 mg of cellulase protein per litre washing solution.

5. A detergent composition according to claim 1, wherein first and second cellulase component are present in a weight ratio of cellulase protein in the range from about 30:1 to about 1:30.

6. A detergent composition according to claim 5, wherein first and second cellulase component are present in a weight ratio of cellulase protein in the range from about 10:1 to about 1:10.

7. A detergent composition according to claim 6, wherein first and second cellulase component are present in a weight ratio of cellulase protein in the range from about 2:1 to about 1:2.

8. A detergent composition according to any of the claims 1-7, wherein the first and the second cellulase component, respectively, is a fungal or bacterial cellulase component.

9. A detergent composition according to claim 8, wherein the first and the second cellulase component, respectively, is one derivable from a strain of Humicola, Bacillus, Trichoderma, Fusarium, Myceliophthora, Phanerochaete, Schizophyllum, Penicillium, Aspergillus, or Geotricum.

10. A detergent composition according to any of the claims 1-9, wherein the first cellulase component exhibits catalytic activity on low molecular weight carbohydrate substrates.

11. A detergent composition according to claim 10, wherein the first cellulase component has a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} of at least 0.01 s^{-1}.

12. A detergent composition according to any of the claims 1-11 wherein the first cellulase component is a core enzyme or a single domain protein.

13. A detergent composition according to any of the claims 1-12 wherein the first cellulase component exhibits low catalytic activity on dyed microcrystalline cellulose and is inadequate or incapable of providing colour clarification.

14. A detergent composition according to any of the claims 1-13, wherein the first cellulase component is a cellobiohydrolase component which is immunoreactive with an antibody raised against a highly purified ~70kD cellobiohydrolase (EC 3.2.1.91) derived from *Humicola insolens*, DSM 1800, or which is a derivative of the ~70kD cellobiohydrolase exhibiting cellulase activity.

15. A detergent composition according to claim 14 wherein the cellobiohydrolase component has the amino acid sequence listed as SEQ ID NO:1 or a variant of said cellobiohydrolase having an amino acid sequence being at least 60% homologous with said sequence.

16. A detergent composition according to any of the claims 1-13, wherein the first cellulase component is an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~50kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a derivative of the ~50kD endoglucanase exhibiting cellulase activity.

17. A detergent composition according to claim 16 wherein the endoglucanase component has the amino acid sequence listed as SEQ ID NO:2 or a variant of said endoglucanase having an amino acid sequence being at least 60% homologous with said sequence.

18. A detergent composition according to any of the claims 1-13, wherein the first cellulase component is an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~58kD endoglucanase derived from *Fusarium oxysporum*, DSM 2672, or which is a derivative of the ~58kD endoglucanase exhibiting cellulase activity.

19. A detergent composition according to claim 18 wherein the endoglucanase component has the amino acid sequence listed as SEQ ID NO:3 or a variant of said endoglucanase having an amino acid sequence being at least 60% homologous with said sequence.

20. A detergent composition according to any of the claims 1-13 wherein the second cellulase component exhibits high catalytic activity on cellodextrins having 6 glucose units (DP6).

21. A detergent composition according to claim 20 wherein the second cellulase component exhibits high catalytic activity on dyed microcrystalline cellulose.

22. A detergent composition according to claim 21, wherein the second cellulase component has a catalytic activity on Red Avicel per 1 mg of cellulase protein higher than 10^{-4} IU.

23. A detergent composition according to any of the claims 20-22 wherein the second cellulase component has a catalytic activity on cellotriose at pH 8.5 corresponding to k_{cat} below 0.01 s^{-1}.

24. A detergent composition according to any of the claims 20-23, wherein the second cellulase component is an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~43kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a derivative of the ~43kD endoglucanase exhibiting cellulase activity.

25. A detergent composition according to claim 24 wherein the endoglucanase component has the amino acid sequence listed as SEQ ID NO:4 or a variant of said endoglucanase having an amino acid sequence being at least 60% homologous with said sequence.

26. A detergent composition according to claim 24 wherein the endoglucanase component comprises an amino acid sequence encoded by the partial DNA sequence listed as SEQ ID NO:5 or a variant of said endoglucanase having an amino acid sequence being at least 60% homologous with said amino acid sequence.

27. A detergent composition according to claim 20, wherein the second cellulase component is an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~60kD endoglucanase derived from *Bacillus lautus,* NCIMB 40250, or which is a derivative of the ~60kD endoglucanase exhibiting cellulase activity.

28. A detergent composition according to claim 27 wherein the endoglucanase component has the amino acid sequence listed as SEQ ID NO:6 or a variant of said endoglucanase having an amino acid sequence being at least 60% homologous with said sequence.

29. A detergent composition according to any of the claims 1-28, wherein the detergent composition is a granular composition.

30. A detergent composition according to claim 29, wherein the granular detergent composition is a compact granular composition.

31. A detergent composition according to any of the claims 1-28, wherein the detergent composition is a liquid composition.

32. A detergent composition according to claim 31 wherein the liquid composition is a heavy duty liquid composition.

33. A detergent composition according to any of the claims 1-32 which additionally comprises one or more enzymes selected from the group consisting of proteases, lipases, esterases, oxidases, peroxidases and amylases.

34. A detergent composition according to claim 31 or 32 wherein the first cellulase component has an improved stability in the presence of protease.

35. A detergent additive comprising a first cellulase component having retaining-type activity and which is capable of particulate soil removal and a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and which is capable of colour clarification wherein at least one of the components is a single (recombinant) component.

36. A method for treating fabrics in a washing machine wherein the fabric is treated with a washing liquor comprising a first cellulase component having retaining-type activity and which is capable of particulate soil removal and a second cellulase component having multiple domains comprising at least one non-catalytic domain attached to a catalytic domain and which is capable of colour clarification, at least one of the cellulase components being a single (recombinant) component.

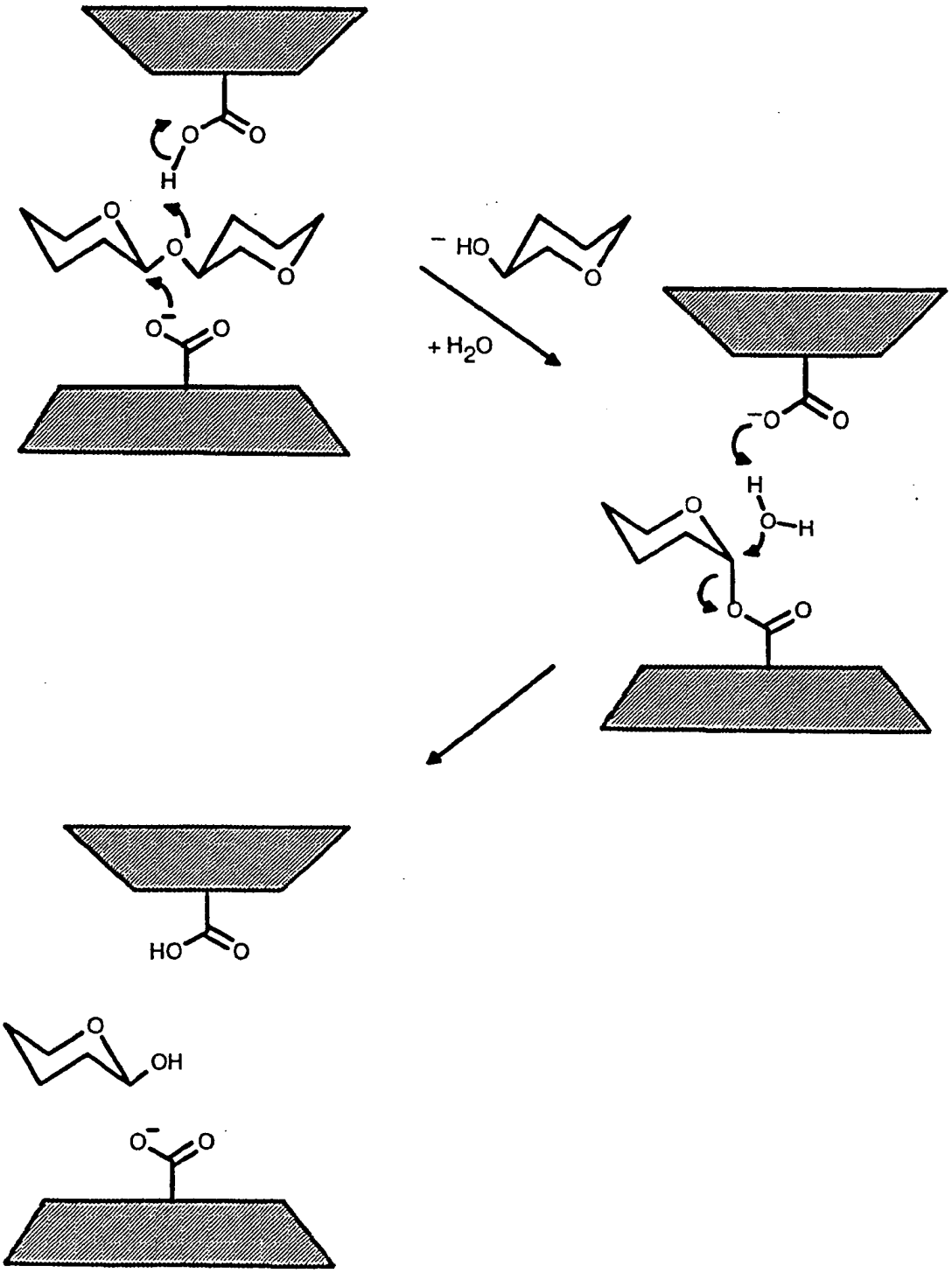

Fig. 1

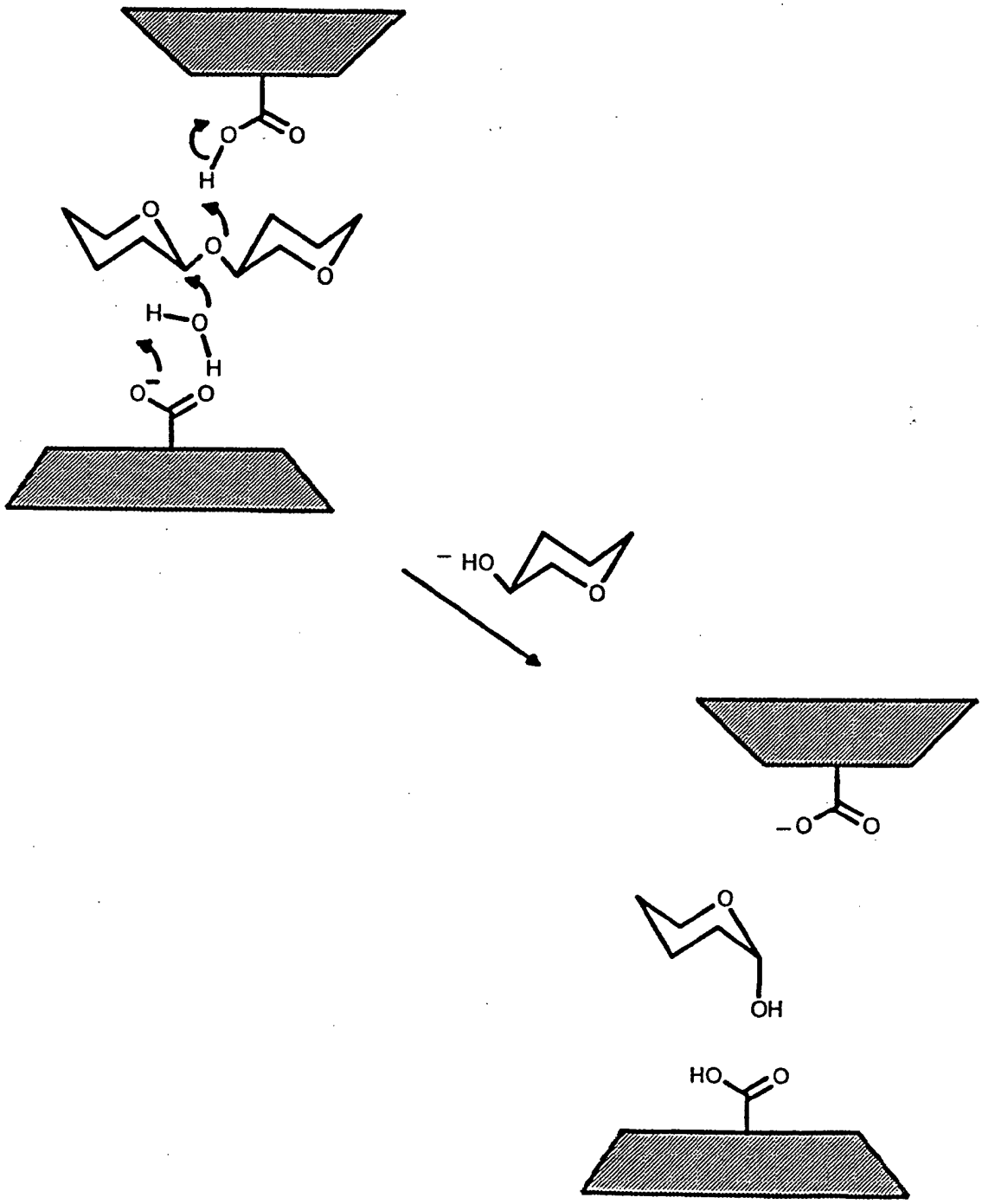

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00280

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C11D 3/386, C12N 9/42
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12N, C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9206165 (GENENCOR INTERNATIONAL, INC.), 16 April 1992 (16.04.92), see pages 1-7, 13-18, page 20, lines 31-36, page 49, lines 31-35 and claim 1	1-36
X	WO, A1, 9206210 (GENENCOR INTERNATIONAL, INC.), 16 April 1992 (16.04.92), see pages 15-22, 27-30 and claims	1-36
X	WO, A1, 9110732 (NOVO NORDISK A/S), 25 July 1991 (25.07.91), see pages 8-16 and claims 31-42, see in particular page 2, line 34 - page 3, line 14 and page 12, lines 19-37	1-36

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 October 1994	25 -10- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00280

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 9117243 (NOVO NORDISK A/S), 14 November 1991 (14.11.91), see pages 1-5	20-25
A	WO, A1, 9117244 (NOVO NORDISK A/S), 14 November 1991 (14.11.91), see pages 1-4 and example 4	14-19
P,X	WO, A1, 9322414 (GENENCOR INTERNATIONAL, INC.), 11 November 1993 (11.11.93)	1-13,20-23, 29-36

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/94

International application No.
PCT/DK 94/00280

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9206165	16/04/92	CA-A- 2093422	06/04/92
		EP-A- 0551408	21/07/93
		JP-T- 6501732	24/02/94
WO-A1- 9206210	16/04/92	CA-A- 2093421	06/04/92
		CA-A- 2093424	06/04/92
		CA-A- 2093428	06/04/92
		EP-A,A- 0551386	21/07/93
		EP-A- 0551394	21/07/93
		EP-A- 0552276	28/07/93
		EP-A,A- 0553280	04/08/93
		FI-A- 931490	31/05/93
		FI-A- 931491	25/05/93
		FI-A- 931495	27/05/93
		JP-T- 6502205	10/03/94
		JP-T- 6502223	10/03/94
		JP-T- 6502226	10/03/94
		US-A- 5246853	21/09/93
		US-A- 5290474	01/03/94
		US-A- 5328841	12/07/94
		WO-A- 9206183	16/04/92
		WO-A- 9206209	16/04/92
		WO-A- 9206221	16/04/92
WO-A1- 9110732	25/07/91	AU-A- 7189491	05/08/91
		EP-A- 0510091	28/10/92
		JP-T- 5503424	10/06/93
WO-A1- 9117243	14/11/91	AU-B- 639570	29/07/93
		AU-A- 7887491	27/11/91
		EP-A- 0531372	17/03/93
		JP-T- 5509223	22/12/93
WO-A1- 9117244	14/11/91	AU-B- 652153	18/08/94
		AU-A- 7791091	27/11/91
		EP-A- 0531315	17/03/93
WO-A1- 9322414	11/11/93	NONE	

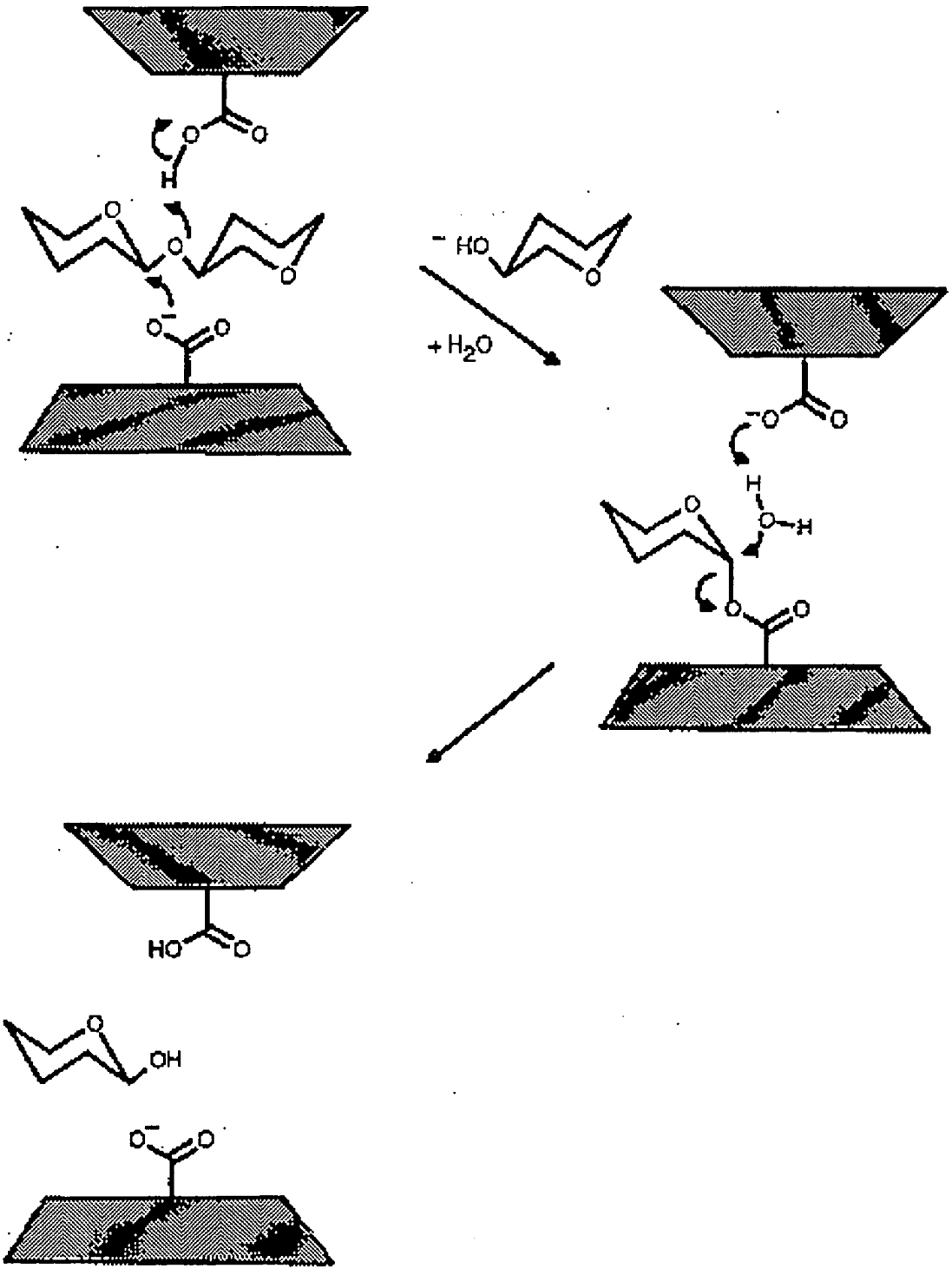

Fig. 1

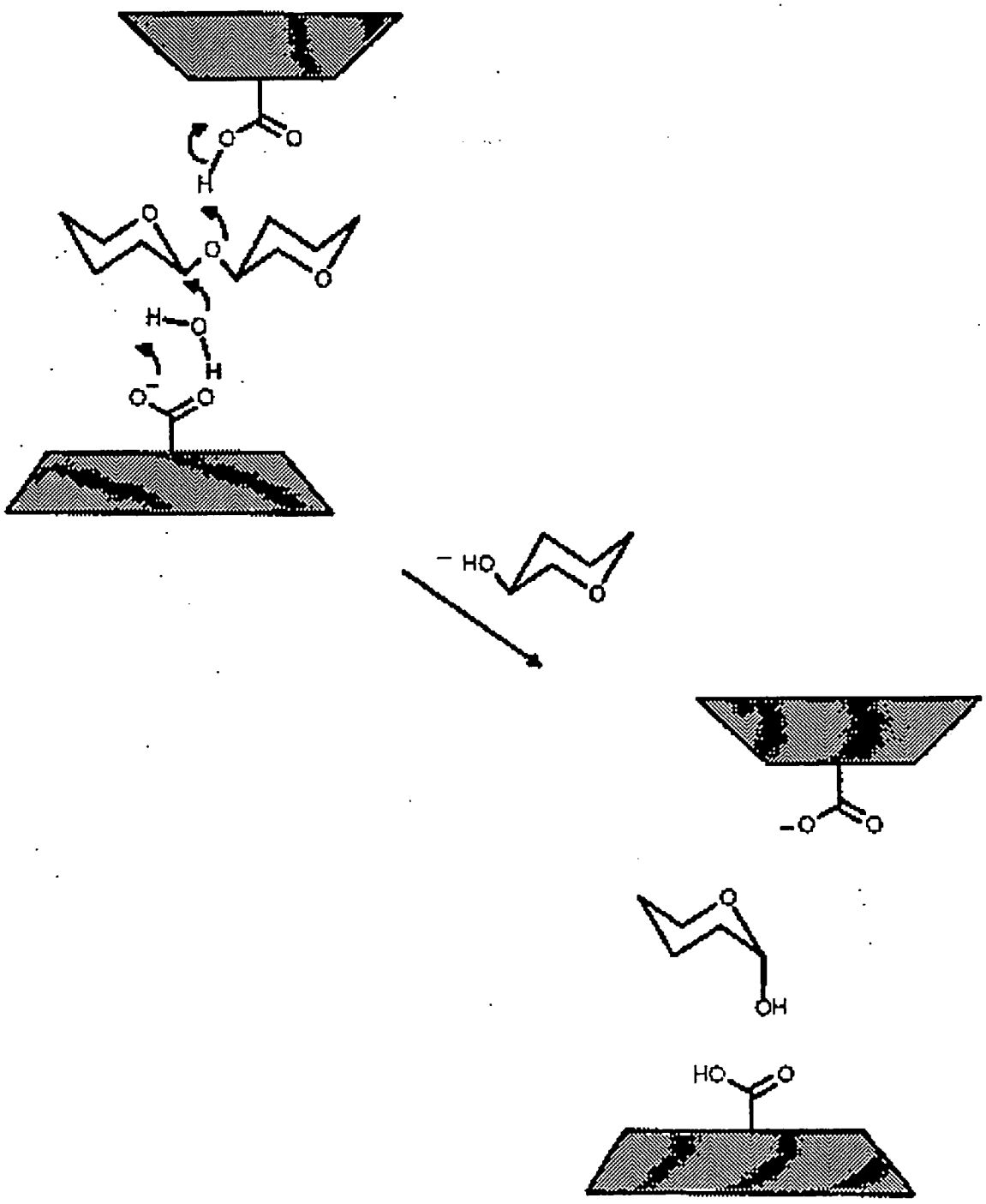

Fig. 2